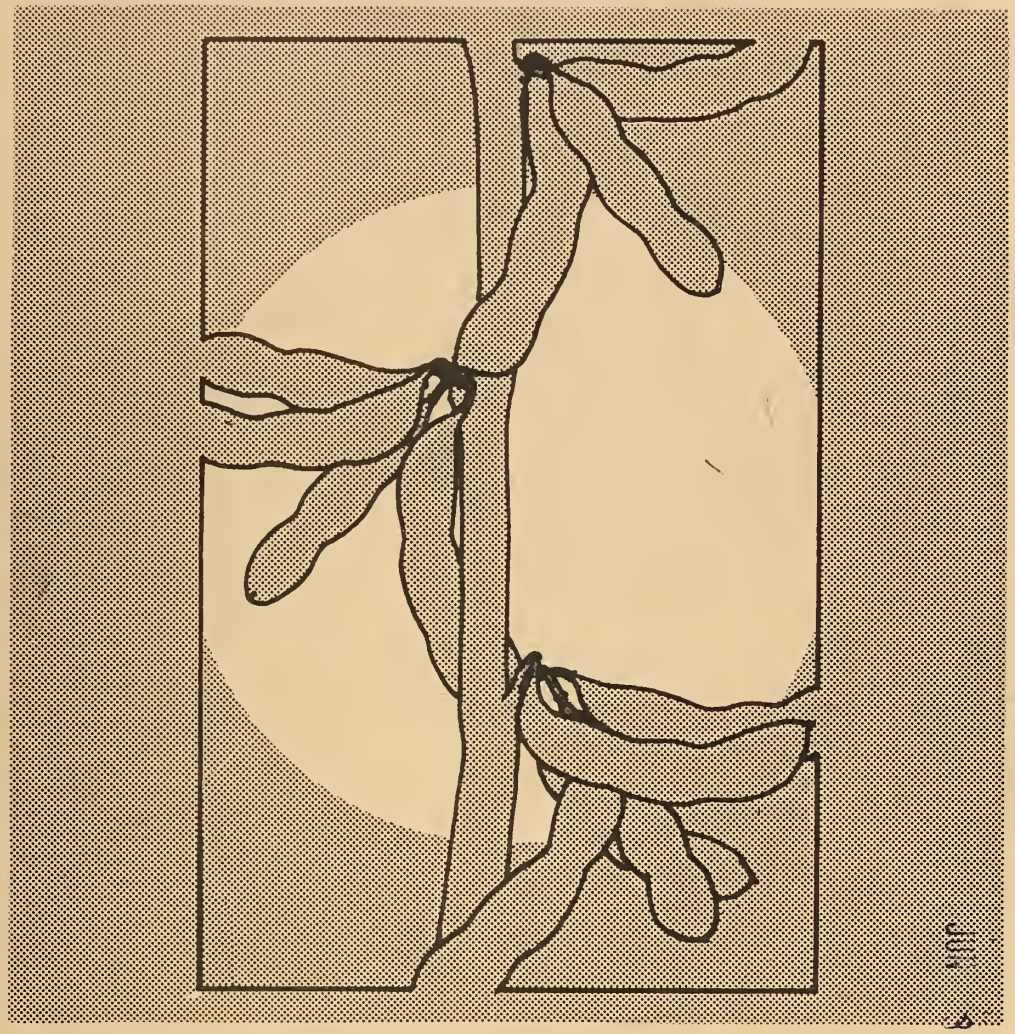
Soybean Genetics Newsletter



Volume 15 APRIL 1988

Agricultural Research Service-USDA
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I. FOREWORD

Once again, for the fifteenth time, the SOYBEAN GENETICS NEWSLETTER offers, to an ever-expanding readership, articles on a wide scope of specialties within the field of soybean genetics, written by authors from all over the world. The continued success of this Newsletter depends almost entirely upon you, the readers and writers. The suggestions--the feedback--that we receive after the printing of each issue affects the content and format of the succeeding issues. Of course, our own preferences shape some of the format. When we seek the address of a specific soybean scientist, we refer to an even-numbered volume of the newsletter for the alphabetical listing of subscribers. To find names of scientists at specific locations, odd-numbered volumes list the subscribers geographically.

We welcome your suggestions for further improvement or more convenient arrangement of the Soybean Genetics Newsletter.

We call your attention to the second biennial CONFERENCE OF THE MOLECULAR AND CELLULAR BIOLOGY OF THE SOYBEAN, to be held at Iowa State University, Ames, July 25-27, 1988. Topics will include: Mechanisms of host-plant interaction; Nitrogen fixation; Genetic variability and diversity; Plant regeneration; Selection at the cellular level; Mechanisms of gene transfer; and Protein and oil synthesis. For further information, contact Randy Shoemaker, G401 Agronomy, Iowa State University, Ames, IA 50011.

Graduate students and technicians who made this volume possible are Laurie Amberger, Sandra Benavente, Susan Blomgren, Mark Chamberlain, Brad Hedges, Holly Heer, Dana Schaulis, Laura Sellner and Zhang Fan. Without their volunteer assistance, this newsletter would not have been possible.

SOYBEAN GENETICS COMMITTEE REPORT - FEBRUARY 1988

Minutes of the Meeting

The Soybean Genetics Committee met February 22, 1988 in conjunction with the Soybean Breeders Workshop in St. Louis, MO.

Committee members in attendance were R. L. Bernard, G. R. Bowers, R. I. Buzzell, X. Delannay, B. A. McBlain and J. H. Orf. Also present were T. E. Devine, G. A. Juvik, B. D. Rennie and D. B. Weaver. T. E. Devine and B. D. Rennie were elected to three-year terms. R. I. Buzzell and X. Delannay were elected ex officio for one year to serve as chairman and to review the assigning of symbols for transformed material, respectively. Current committee members and the expiration date of their terms are as follows:

R. L. Bernard (Ex officio)
(Curator of soybean genetics collection)
Department of Agronomy
University of Illinois
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G. R. Bowers (1990)
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R. I. Buzzell, Chairman (Ex officio,1989)
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T. E. Devine (1991)
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Y. T. Kiang (1989)
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B. D. Rennie (1991)
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Procedure: As in the past, manuscripts concerning qualitative genetics interpretation and gene symbols should be sent to the chairman for review.

In order to facilitate the review process, the Committee will proceed as follows:

1. The review will only be for "validity of the genetic interpretation" and "appropriateness of gene symbol". The manuscripts will not be reviewed for style except as it influences the clarity of interpretation and it will not be given a "peer review". Authors may submit unpolished (but comprehensible) manuscripts for review. This should reduce some of the delay involved in publishing a paper.

2. Reviewers of manuscripts will be given a deadline of two weeks to return the reviewed manuscript to the Chairman (who will then give it to the author as soon as possible). If the reviewer has not returned the manuscript by this time (or phoned in their comments), a phone call will be made to remedy the situation. If authors have not received a reply within two months of submission, they should contact the Chairman.

Assignment/Approval of Gene Symbols: If gene symbols are being assigned in genetic studies where the material is from induced mutants, variants from heterogeneous populations, or from transgenic changes, then the authors should deposit representative genetic material in the Genetic Type Collection. Dr. R. L. Bernard is Curator for all maturity groups. A form for this is on page 27.

Gene symbols will only be approved in cases where the relevant material is available in one of the soybean germplasm collections for distribution to researchers. The Committee encourages authors not to assign any symbol when they are doing genetic work on material that will not be made available. (Publication of genetic interpretations does not depend on symbols, in most cases). The purpose of assigning a symbol is to ensure constancy when others use the material for subsequent studies. If the material is not made available, a symbol is unnecessary.

Summaries for the Past Year: A list of the soybean gene symbols approved March 1987-February 1988 is given in Table 1. A list of new linkage groups is given in Table 2. Previously approved genes that have been recently published are given in Table 3. A list of unapproved gene symbols that have appeared tentatively in publications recently is given in Table 4; these symbols should NOT be used until they are assigned in genetic studies. Errata concerning recently published gene symbols is given in Table 5.

It was noted that, in 1982, gene symbols Rcs4 and rcs4 were published in Brazil for resistance and susceptibility to frogeye leafspot caused by race 4 Cercospora sojina (3). These were valid gene symbols; however, researchers working with frogeye leafspot should note that allelism tests, apparently, have not been run to determine whether the three genes, Rcs1, Rcs2 and Rcs4, are at different loci. Rcs4 occurs in Davis and Santa Rosa, and rcs4 occurs in Hood and Roanoke.

R.I. Buzzell
Chairman

TABLES FOR SOYBEAN GENETICS COMMITTEE REPORT FOR MARCH 1987-FEBRUARY 1988

Table 1. Soybean gene symbols approved March 1987-February 1988

Date	Authors	Trait	Genes
Apr 30,1987	Chiang, Gorman & Kiang	PGI isozymes	Pgi1-a Pgi1-b pgi1 Pgi2 pgi2 Pgi3-a Pgi3-b
Jun 1,1987	Frankenberger, Wilcox & Cavins	Palmitic acid	Fap1 fap1 Fap2 fap2
Jun 2,1987	Doong & Kiang	Endopeptidase isozyme	Enp-c
Jul 23,1987	Doong & Kiang	Fluorescent esterase isozyme	Fle fle
Dec 3, 1987	Reese & Boerma	Green seedcoat	G2 g2 g3 G3
Jan 21,1988	Skorupska & Palmer	Male sterile	ms1 (Danbury) ms3 (Plainview) ms4 (Fisher)
Feb 5,1988	Bult & Kiang	Esterase isozyme	Est-a Est-b
Feb 15,1988	Honeycutt, Burton, Shoemaker & Palmer	Shrivelled seed	Shr shr
Feb 16,1988	Palmer, Bernard &	Chl-deficient	Y19 y19 Y22 y22 Y23 y23
Feb 29,1988	Chiang & Kiang	MPI isozymes	Mpi-d mpi

Table 2. New soybean linkage groups March 1987-February 1988

Date	Authors	Linkage group		
Apr 30,1987	Chiang, Gorman & Kiang	16	*Pgi* and *Pgd1*	15.3±0.7%R
May 1,1987	Rennie, Zilka, Cramer & Beversdorf	17	*Fan* and *Idh2*	28.2±2.7%R
Jan 27,1988	Thorson, Hedges & Palmer	14	*Y9* and *Pb*	27.3±1.1%R

Table 3. Previously approved genes that have been recently published

Gene	Strain	Phenotype	Reference
Aco1-a	Evans	Aconitase band 1 least mobile variant	5
Aco1-b	PI 257430	Most mobile	
Aco2-a	PI 464918	Aconitase band 2 least mobile variant	5
Aco2-b	Evans;cv	Most mobile	
Aco3-a	Evans;cv	Aconitase band 3 least mobile variant	5
Aco3-b	PI 342622	Most mobile	
Aco4-a	Williams;cv	Aconitase band 4 least mobile variant	5 & 10
Aco4-b	Harosoy;cv	Most mobile	5 & 10
Aco4-c	Minsoy	Intermediate mobility	5
Df6	C1421;cv	Normal plant height	13
df6	EMS>MS2060	Dwarf	
E5	PI 90.837	Later maturity	8
e5	Harosoy	Normal maturity	
Enp-a	Altona;cv	Endopeptidase isozyme Rf 0.23 band	5 & 11
Enp-b	Harosoy;cv	Rf 0.29 band	5 & 11
Fan	Century	Normal level of linolenic acid in seed	14
fan	EMS>C1640	Lower level of linolenic acid in seed	
Hs1	Williams	Sensitivity to sulfonylurea herbicides	12
hs1	ENU>mutant	Enhanced tolerance	
Hs2	Williams	Sensitivity to sulfonylurea herbicides	12
hs2	ENU>mutant	Enhanced tolerance	
Hs3	Williams	Sensitivity to sulfonylurea herbicides	12
hs3	ENU>mutant	Enhaced tolerance	
Rbs1	L78-4094	Resistant to brown stem rot	6
rbs1	LN78-2714	Susceptible	
Rbs2	PI 437833	Resistant to brown stem rot	6
rbs2	Century	Susceptible	
Rdc1	Tracy-M	Resistant to stem canker	7
rdc1	J77-339	Susceptible	
Rdc2	Tracy-M	Resistant to stem canker	7
rdc2	J77-339	Susceptible	

> = mutant induced by the chemical mutagen

Table 4. Tentative symbols that have NOT been approved for use as genes

Symbol	Strain	Reference
Rps1-d	PI 103091*	9
Rps3-d	PI 82312N**	1 & 9
Rps3-e	PI 273483D#	1 & 9
Rps 7	PI 82312N**	9
rps 7	Harosoy	9

* PI 103091 has one or more Rps genes; but, no published results.

** PI 82312N probably has Rps3-b and Rps5 (2), i. e., there is no evidence that Rps3-d and Rps7/rps7 exist as previously described.

\# PI 273483D has Rps1-c and probably an allele at Rps3 (2).

Table 5. Errata for gene symbols

Reference (10):	In Fig. 1 and Table 1, Aco1-a and Aco1-b should read Aco4-a and Aco4-b.
Reference (4):	Aco4-b should read Aco4-c; Aco4-c should read Aco4-b in order to agree with references (5) and (10). The variant of least mobility is Aco4-a (found in Williams), the variant of intermediate mobility is Aco4-c (found in Minsoy), and the variant with greatest mobility is Aco4-b (found in Harosoy).

References

1. Athow, K. L. 1987. p. 701 in Soybeans: Improvement, Production and Uses.

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4. Doong, J. H. and Y. T. Kiang. 1988. Genome 29:713-717.

5. Griffin, J. D. and R. G. Palmer. 1987. Crop Sci. 27:885-892.

6. Hanson, P. M., S. A. Sebastian, C. D. Nickell and L. E. Gray. 1988. Crop Sci. 28:

7. Kilen, T. C. and E. E. Hartwig. 1987. Crop Sci. 27:863-864.

8. McBlain, B. A. and R. L. Bernard. 1987. J. Hered. 78:160-162.

9. Palmer, R. G., R. C. Shoemaker and B. Rennie. 1987. Soybean Genet. Newsl. 14:41-58.

10. Rennie, B. D., W. D. Beversdorf and R. I. Buzzell. 1987. J. Hered. 78:323-326.

11. Rennie, B. D., W. D. Beversdorf and R. I. Buzzell. 1987. J. Hered. 78:327-328.

12. Sebastian, S. A. and R. S. Chaleff. 1987. Crop Sci. 27:948-952.

13. Werner, B. K., J. R. Wilcox and T. L. Housely. 1987. Crop Sci. 27:665-668.

14. Wilcox, J. R. and J. F. Cavins. 1987. J. Hered. 78:410.

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USDA Northern Soybean Germplasm Collection Report

February, 1988

In 1987, a total of 355 seed requests for a total of 5,590 seedlots were filled from the USDA Northern Soybean Germplasm Collection. Of this total, there were 284 requests for 4,402 seedlots from 34 U. S. states and 71 requests for 1,188 seedlots from 31 foreign countries.

During the summer of 1987, 1,057 4-row plots were grown at Urbana for soybean maintenance. A total of 112 new accessions were grown, of which 4 were genetic types and 108 were PI strains (1 Taiwan, China; 46 South Korea; 2 Japan; 59 Soviet Union). In addition, 511 accessions were added to the collection after the 1987 harvest and are available for distribution.

The number of accessions maintained at Urbana is listed below by maturity group and country of origin.

Maturity group	Genetic type	Isolines	Post-1944 public varieties	Old varieties	FC Strains	PI Strains	1987 PI additions	PI Total	Grand Total	Percentage
000	0	0	1	3	1	92	6	98	103	1.2
00	0	0	15	5	4	340	0	340	364	4.4
0	0	0	20	7	6	824	14	838	871	10.5
I	8	11	26	23	3	1121	12	1133	1204	14.6
II	17	94	42	26	6	1235	74	1309	1494	18.1
III	30	15	36	38	13	1066	141	1207	1339	16.2
IV	49	186	40	38	18	2297	264	2561	2892	35.0
Total	104*	306	180	140	51	6975	511	7486	8267*	100.0
China				65	6	1273	1	1274	1345	17.5
Japan				35	10	1035	509	1544	1589	20.7
Korea				12	0	2024	1	2025	2037	26.5
USSR				6	0	1807	0	1807	1813	23.6
Other Asian				0	0	29	0	29	29	.4
Europe				3	0	762	0	762	765	10.0
USA/Canada				18	34	0	0	0	52	.7
Other**				1	1	15	0	15	17	.2
Unknown				0	0	30	0	30	30	.4
Total				140	51	6975	511	7486	7677	100.0

* There are also 4 T strains of Maturity group V (2) and VII (2).

** Africa, Australia, and Latin America.

There were 186 new wild soybean accessions grown at Urbana (57 Japan, and 129 Soviet Union) and 47 at Stoneville, Mississippi (1 South Korea and 46 Japan). One accession was added to the wild soybean collection this year bringing the total number available for request to 678.

Country of Origin	Number of accessions by Maturity Group													Total
	000	00	0	I	II	III	IV	V	VI	VII	VIII	IX	X	
China	11	11	21	12	31	13	17	8	9	1	0	0	0	134
China, Taiwan	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Japan	0	0	0	0	0	1	7	52	85	46	0	3	0	194
South Korea	0	0	0	0	2	0	40	244	27	1	0	0	0	314
USSR	0	17	7	5	5	0	0	0	0	0	0	0	0	34
Total	11	28	28	17	38	14	64	304	121	48	0	3	2	678

The second and final year of Soybean Germplasm Evaluation 4 was completed in 1987. A total of 510 PI accessions from 446.893 to 486.355 and 38 varieties released since 1983 were evaluated for 31 descriptors. Dr. James Orf grew maturity groups 000, 00, and 0 (62 lines plus 12 checks) at the University of Minnesota. Maturity groups I to IV (486 lines plus 26 checks) were evaluated at Urbana, Illinois.

Volume 1 of the USDA Soybean Germplasm Collection Inventory was published in 1987 in conjunction with the International Soybean Program (INTSOY). This volume contains entries for 204 domestic cultivars introduced or developed by 1947, 90 strains identified by FC numbers, and 1383 foreign cultivars and 5 wild soybeans introduced prior to 1945 and identified by PI numbers less than 150.000. Volume 2 of the USDA Soybean Germplasm Collection Inventory, expected to be published in 1988, will include all soybean and wild soybeans introduced from 1945 to 1985 (PI numbers 150.000 to 500.000).

Nearing completion as a USDA Technical Bulletin is the "Origins and Pedigrees of Public Soybean Varieties in the United States and Canada". This publication will include origin information on 204 domestic cultivars introduced or developed by 1947 and 236 public cultivars developed and released from 1947 to 1986.

The perennial species of Glycine (i.e., subgenus Glycine) are now being maintained by Theodore Hymowitz at the University of Illinois under the auspices and support of the USDA. The entries consist of the following groups of additions:

	A PI 193.232 to 429.809* 1950-78	B PI 499.904 to 499.952 1985	C Australian Core Collection PI 509.448 to 509.502 1987	D Hymowitz Collection PI 440.927 to 505.304 1980-86	Total	Re-classified total
G. arenaria	0	0	3	0	3	6
G. argyrea	0	0	3	1-1	3	3
G. canescens	1	4	15	34-8	46	46
G. clandestina	11	6	17	39-6	67	55
G. curvata	0	0	4	4-3	5	5
G. cyrtoloba	1	0	5-1	13-3	15	18
G. falcata	2	2	8-1	3-2	10	10
G. latifolia	6	0	6	5-2	16	16
G. latrobeana	0	0	9-1	9-4	11	11
G. microphylla	1	0	16-3	1-1	9	18
G. tabacina	27	11	20-2	68-4	115	117
G. tomentella	17	20	0	172-14	213	210
G. species?	0	6		0	6	7
	66	49	111-8	349-48	519 +3 sub-lines	522

A. These strains have been maintained and distributed by USDA at the University of Illinois since their introduction in 1950 to 1978.

B. This group was received in 1985 from Dr. J. R. Lawn, CSIRO, Queensland, Australia. It was collected in Australia independently of C and is now stored at NSSL.

C. This group was received in 1986 from Dr. A. H. D. Brown, CSIRO, Canberra, and is his choice from his collection of over 600 accessions to represent the diversity of the subgenus. The first number is the total in the core collection. The number after the minus sign tells how many of these are in the A group.

D. The first number is the total. The number after the minus sign tells how many of these are in the C group.

*PI 433.361 and 433.376 were duplicates and were discarded.

Annually updated checklists and other printed information are available from the curator. These include:

1. U. S. and Canadian Germplasm Variety Checklist (140 strains with maturity group and descriptive code), January 1987.

2. U. S. and Canadian Public Variety Checklist (173 strains with maturity group and descriptive code), June 1987.

3. FC and PI Strain Checklist (7,536 strains with maturity group), February 1988.

4. Wild Soybean Checklist (675 strains with maturity group), January 1986.

5. Wild Soybean Inventory (675 strains with maturity group and passport data), January 1986.

6. Genetic Type List (112 strains, up to T292H, with genotype, phenotype, and origin information), February 1988.

7. Genetic Isoline (300 strains with genotype, phenotype, and origin information), March 1975.

R. L. Bernard, curator
G. A. Juvik, assistant curator

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USDA SOUTHERN SOYBEAN GERMPLASM REPORT

Report on germplasm in Maturity Groups V to X - February, 1988

Maturity	Total entries 1983	Total entries 1984	Total entries 1985	Total entries 1987
V	1520	1549	1550	1550
VI	470	482	486	487
VII	334	346	349	351
VIII	285	297	303	308
IX	124	131	143	149
X	151	154	158	165
	2884	2959	2989	3010

671 new additions were pure lined in 1987. Seed will be available after increase in 1988. Will be added to GRIN 1988-89.

Stoneville will grow 304 lines of Germplasm VII in 1988 for increase.

Stoneville will grow 30 lines of 4 plants each for single row selection in 1988.

1987 seed requests filled: 4,902 packets, to 20 countries, and to 26 states.

E. Hartwig
C. J. Edwards, Jr.

SOYBEAN GERMPLASM CROP ADVISORY COMMITTEE REPORT

The Soybean Germplasm Crop Advisory Committee (CAC) held its annual meeting February 22, 1988 at the Soybean Breeders' Workshop in St. Louis, MO. Eleven of the 14 members were in attendance. Also attending were: Mark Bohning, CAC Facilitator; Gail Juvik and Calton Edwards, assistant curators of the northern and southern germplasm collection, respectively; R. I. Buzzell, public breeder, invited observer from Canada; and John Belt, GRIN observer. Those elected to three-year terms were: Lavone Lambert, USDA-ARS, Stoneville, MS; James Orf, University of Minnesota; and Reid Palmer, USDA-ARS, Iowa State University.

Updates on both the northern and southern portions of the USDA Soybean Germplasm Collection were given by Richard Bernard, Gail Juvik, Calton Edwards, and Edgar Hartwig. Richard Bernard also reported on the status of the collection of perennial Glycine species, with special emphasis upon the problems involved in increasing seed of these species. Dr. T. Hymowitz has assumed the major responsibility of maintenance and seed increase of the perennial Glycine species. Greenhouses have been used for the major part of the year to maintain these accessions. Field areas that can be covered to avoid early and late frost have allowed production for many of the entries, thus saving valuable greenhouse space. Reports on the current germplasm collections are presented in the Soybean Genetics Newsletter (SGN) pages 9-13.

The availability of soybean germplasm data from the Germplasm Resources Information Network (GRIN) was discussed. Mark Bohning reported that over 200 people are now using the GRIN system. User manuals and codes for accessing the soybean germplasm data base in GRIN are available by a request to: John Belt, USDA-ARS, Bldg. 001, Rm 132B, BARC-West, Beltsville, MD 20705.

The Soybean Germplasm CAC bylaws were published in the SGN 13:31-32, 1986. A question was raised concerning the length of service for the chairman and vice-chairman of the committee. It was suggested that revisions be sent to the chairman, shared with committee members, and that a proposal for changing the bylaws be acted upon at the 1989 meeting.

Additions to the northern collection include 511 new accessions, bringing the total to 8,265. Greatest numbers of accessions are from Korea, USSR, Japan, and China, in that order. Volume I of USDA Soybean Germplasm Collection Inventory, INTSOY Series Number 30, was published in August, 1987. Volume II is to follow in 1988. The wild soybean collection has 678 accessions. The southern collection has 3,010 accessions with an additional 671 expected to be added to the working collection in 1989. Total numbers of accessions requested in 1987 were less than in previous years, but the number of scientists making requests was more than in previous years. Requests are becoming more specific and selective, with few requests for all accessions within a maturity group. The curators ask scientists requesting germplasm to please specify the purpose for which it is to be used, and also to share with the curators any information that might help to catalogue the accessions more precisely.

Randall Nelson reported on potential opportunities for soybean germplasm exchange and research cooperation with the People's Republic of China. Over 600 soybean cultivars have been listed in "A Catalogue of Chinese Crop Germplasm for Exchange." The committee recommended that an immediate request for these cultivars be made through the office of Henry Shands or George White. Publicly developed United States and Canadian cultivars should be offered to the Chinese with this request.

Richard Wilson reported that there is an increased interest in vegetable or specialty soybeans for niche markets. He pointed out the desirability of some method (in addition to seed size) of identifying accessions having a potential for vegetable or specialty markets.

Thomas Kilen reported that the soybean germplasm groups V through X were being systematically evaluated for resistance to cyst nematodes, foliar feeding insects, and selected diseases (stem canker, aerial web blight, and phytophthora rot). In the first screening of about 400 lines for stem canker, about 60% were resistant. Lavone Lambert reported that all of the southern soybean collection would be screened for resistance to soybean looper (SBL) and velvetbean caterpillar. In 1987, 1550 group V accessions were evaluated for SBL resistance. They will be evaluated again in 1988, along with other maturity groups.

Thomas Kilen distributed copies of a draft on "Status of Crop Vulnerability" prepared from responses on the subject by members. These thoughts on genetic diversity and crop vulnerability were written to address questions raised in a 24 April 1987 memo from Henry Shands. The majority of the members believe that the maintenance of maximum diversity within the germplasm collections is critical to resource development and utilization. Evaluating and cataloging traits in the collection is very important so that material held in reserve can be quickly introduced into the commercially used gene pool when needed.

Donald Schmitt led a discussion on problems encountered in soybean cyst nematode (SCN) research. Of particular concern is a need to define more precisely what constitutes plant resistance, and to define SCN races. A motion was made to form an ad hoc committee to develop for the 1989 Soybean Workshop a half-day session to provide information on standardizing approaches and techniques to better define SCN races and plant resistance to SCN. Donald Schmitt agreed to chair the committee.

Meeting time expired before all agenda items were discussed. Inputs were requested from Soybean CAC members concerning the topics of: 1) identification of high priority research approaches for germplasm enhancement, 2) CAC position on germplasm patenting, 3) CAC position on limiting the number of closely related germplasm lines to be registered, and 4) impact of a soybean marketing system where value would be based on the protein and oil content.

Following are the current committee members, addresses, areas of representation, and years of service:

Name	Address	Area of representation	Years of service
T. Scott Abney	USDA, ARS Dept. of Plant Pathology Purdue University W. Lafayette, IN 47907	Plant Pathology	1986-1989
R. L. Bernard	USDA, ARS Department of Agronomy University of Illinois 1102 South Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex officio
Edgar E. Hartwig	USDA, ARS Soybean Prod. Research P. O. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Kuell Hinson	USDA, ARS Dept. of Agronomy University of Florida IFAS Bldg. 63 Gainesville, FL 32611	Public Breeding South	1984-1987 1987-1990
Clark Jennings	Pioneer Hi-Bred Int'l 3261 W. Airline Hwy. Waterloo, IA 50703	Private Breeding North	1984-1987 1987-1990
Thomas C. Kilen	USDA, ARS Soybean Prod. Research P. O. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Philip Miller	USDA, ARS Beltsville Agric. Res. Center Bldg. 005, BARC-W Beltsville, MD 20705	USDA National Program Staff	ex officio
Randall Nelson	USDA, ARS Dept. of Agronomy University of Illinois 1102 South Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex officio

Current committee members (Continued)

Name	Address	Area of representation	Years of service
J. H. Orf	Dept. of Agronomy and Plant Genetics University of Minnesota St. Paul, MN 55108	Public Breeding North	1985-1988 1988-1991
Reid G. Palmer	USDA, ARS G301 Agronomy Iowa State University Ames, IA 50011	Cytogenetics and Molecular Genetics	1985-1988 1988-1991
Donald P. Schmitt	Dept. of Plant Pathology Box 7631 North Carolina State Univ. Raleigh, NC 27695-7631	Nematology	1987-1990
J. Grover Shannon	Asgrow Seed Company P. O. Box 210 Marion, AR 72364	Private Breeding South	1986-1989
Richard Wilson	4114 Williams Hall North Carolina State Univ. Raleigh, NC 27695-7620	Physiology	1983-1986 1986-1989
Lavone Lambert	USDA, ARS Soybean Prod. Research P. O. Box 196 Stoneville, MS 38776	Entomology	1988-1991

Thomas Kilen was reelected as chairman of the committee and Clark Jennings was reelected vice-chairman. Both will serve one-year terms.

Thomas C. Kilen, Chairman
Soybean Germplasm Crop
Advisory Committee

USDA SOYBEAN GENETIC TYPE COLLECTION

The Soybean Genetic Type Collection is a part of the USDA Soybean Germplasm Collection and is composed of strains used in qualitative genetic studies that are not in the Collection as domestic varieties or FC or PI numbers. These strains are assigned T-numbers in chronological order as submitted to the Collection. Where previous strain designations have been used, they are given in parentheses under the T-number. Most of the T-strains are mutants. Those that are segregants from crosses or presumed outcrosses are marked with * after the genotype. For T-strains with an H suffix (e.g., T 211H) the allele is carried as the heterozygote because the homozygote is lethal, sterile, or very weak. For T-strains with a M suffix (e.g., T 225M) the trait is maintained by selecting the mutable genotype. Cytoplasmically inherited traits are prefixed by *cyt-*. Genes for secondary traits of interest are listed in parentheses. Numerical superscripts are used to indicate backcrosses; e.g., Lincoln^2 x Richland means Lincoln x (Lincoln x Richland).

Strain	Genotype	Phenotype	Parental origin	When and where found
T16	--*	Brown hilum on black seed	Ebony	Before 1930
T31	*p2*	Puberulent	Soysota x Ogemaw	1926 by F.W. Wentz at Ames, Iowa
T 41	*ln (d1 d2)**	Narrow leaflet	Unknown	Before 1930 at Urbana
T 43 (Progeny 435B)	*P1 (cyt-G1)**	Glabrous	Medium Green x "glabrous"	Before 1927 at Urbana
T 48	--*	Spread hilum	Manchu x Ebony	Before 1930 at Urbana
T 54	*dt1**	Determinate stem	Manchu	Before 1927 at Urbana
T 93	*v1 (D1 d2 or d1 D2)*	Variegated leaves	Hybrid population	Before 1931 at Urbana
T 93A	*v1 (d1 d2)*	Variegated leaves	T 93	At Urbana
T 102	*y4 le*	Greenish yellow leaves weak plant; seed lectin absent	Wilson-Five	Before 1932 at Urbana

Strain	Genotype	Phenotype	Parental origin	When and where found
T 104	*d1 d2 G cyt-G1**	Green seed embryo, green seed coat	T 42 (green cotyledon from H. Terao) x "Chromium green"	Before 1932 at Urbana
T 116H	*y5*	Greenish yellow leaves, very weak plant	Radium-treated PI 65.388	Before 1934 at Urbana
T 117 (L34-602)	*Dt2 1w1 Lw2**	Semi-determinate stem, non-wavy leaf	AK114 x PI 65.394	Before 1934 at Urbana
T 122	*l0 (d1 d2)*	Oval leaflet, few-seeded pods	Unknown	Before 1934 at Urbana
T 134	*y5*	Greenish yellow leaves	Illini x Peking	1937 at Urbana
T 135	*y9*	Bright greenish yellow leaves	Illini	1938 at Urbana
T 136	*y6 (1n dt1)*	Pale green leaves	PI 88.351 x Rokusun	1937 at Urbana
T 138 (L35-1156)	*y7 y8*	Yellow growth in cool weather	Unknown	Before 1935 at Urbana
T 139	*g y3**	Yellow seed coat, leaves turn yellow prematurely	Illini	About 1936 by Brunson in Kansas
T 143	*Lf1 g y3 y7 y8**	5-foliolate, leaves turn yellow prematurely, and in cool weather	T 138 x T 137, (T 137 is *y3* from a cross in PI 81.029)	By 1935 at Urbana
T 144	*d1 d2 v1 y7 y8**	Green seed embryo, variegated leaves, yellow growth in cool weather	LX431: T 93A x T 138	At Urbana
T 145 (9-776)	*P1**	Glabrous	Unknown	At Urbana
T 146	*r-m**	Brown seed with black stripes	LX286: PI 82.235 x PI 91.073	At Urbana
T 152	*i*	Self dark seed coat	Lincoln	By 1938 at Urbana
T 153	*k1*	Dark saddle on seed coat	Lincoln	By 1938 at Urbana

Strain	Genotype	Phenotype	Parental origin	When and where found
T 157	*i*	Self dark seed coat	Richland	By 1938 at Urbana
T 160	--	Pale green leaves	Hahto (Michigan)	By 1938 at Urbana
T 161	*y10*	Greenish yellow seedling	L36-5 from Mandarin x Mansoy	1940 at Urbana
T 162	*y17*	Light yellowish-green leaves	Mandarin	1940 at Urbana
T 164	--	Slightly variegated leaves	Morse	1941 at Urbana
T 171	--*	Long peduncle	Unknown	At Urbana
T 173	*f (ln)**	Fasciated stem	Keitomame *(f)* x PI 88.351 *(ln)*	At Urbana
T 175	*E1 t**	Late maturity, gray pubescence	Unknown	At Urbana
T 176	*1w1 1w2 (Dt2)**	Wavy leaf	Unknown	At Urbana
T 180 (L46-1741-2)	*Rj1**	Nodulating	Same F3 plant as T 181	At Urbana
T 181 (L46-1743-2)	*rj1**	Nonnodulating	Lincoln[2] x Richland	At Urbana
T 201	*rj1**	Nonnodulating	LX1277: L46-1743 x L46-1741	At Ames, Iowa
T 202	*Rj1**	Nodulating	Sib of T201	At Ames, Iowa
T 204 (L48-101)	*1n 10**	Narrow leaflet, 4-seeded pods; oval leaflet, few-seeded pods	T 136 x T 122	At Urbana
T 205 (L48-163)	*1w1 1w2**	Wavy leaf	Dunfield x Manchuria 13177	At Urbana
T 208 (Ind. Acc. 2300-2)	*Se**	Pedunculate inflorescence, small seeds	PI 196.176#('Yu Tae' from Korea)	

Not in Stoneville Collection, considered a duplicate of PI 196.177 (V).

Strain	Genotype	Phenotype	Parental origin	When and where found
T 209 (L50-155)	--*	Dwarf	Lincoln x "wild dwarf"	At Urbana
T 210 (L49-738)	*df2*	Dwarf	Colchicine-treated Lincoln	At Urbana
T 211H (CX3941-844-2-5)	*pm*	Dwarf, crinkled leaves, sterile	Kingwa x T 161	At Lafayette, Indiana
T 216 (L46-266)	--*	Reddish black seeds	PI 86.038 x PI 88.351	1946 at Urbana
T 218M	*Y18-m*	Chlorophyll chimera, (resembles T 225M)	Illini	1952 at Urbana
T 219H (A691-1)	*y11*	Lethal yellow, (heterozygote has greenish yellow leaves)	Richland x Linman 533	1941 at Ames, Iowa
T 220 (L46-431)	--	Greenish yellow leaves	Lincoln	At Urbana
T 221 (L46-426)	--	Yellowish green leaves	Peking	At Urbana
T 223 (L46-429)	--	Yellowish green leaves	Richland	At Urbana
T 224 (L46-428)	--	Greenish yellow leaves	Richland	At Urbana
T 225M	*Y18-m*	Unstable gene resulting in chlorophyll chimera	Lincoln	Before 1955 in Iowa
T 225H	*y18*	Near-lethal yellow leaves	T 225M	
T 226	--	Greenish yellow leaves	Lincoln	1943 at Ames, Iowa
T 227	--	Greenish yellow leaves becoming green	Illini	1943 at Kanawha, Iowa
T 229	*y14*	Light green leaves	F4 Richland x Linman 533	1943 at Ames, Iowa
T 230 (A43K-643-1)	*y13*	Whitish green seedling, greenish yellow leaves	Mandell x Mandarin (Ottawa)	1944 at Kanawha, Iowa

Strain	Genotype	Phenotype	Parental origin	When and where found
T231 (A49-8414)	--	Greenish yellow leaves, weak plant	AX3015-55: Richland x Linman 533	1943 at Ames, Iowa
T232	--	Yellowish green leaves	Hawkeye	1950 at Ames, Iowa
T233	*y12*	Whitish primary leaves, yellowish green leaves	Hawkeye	1950 in field N2100 at Ames, Iowa
T234	*y15*	Pale yellowish green leaves	L46-2132 (Clark progenitor)	1952 at Ames, Iowa
T235 (L58-274)	*wm*	Magenta flower	Harosoy	1957 at Urbana
T236 (L46-232)	*(Lf1 ln y6)**	Red-buff seed	T143 x "*y6 ln pc dt1 w1*"	1946 at Urbana
T238 (S57-3416)	*k3*	Dark saddle on seed coat	X-rayed Clark	1956 at Columbia, Missouri
T239 (L63-365)	*k2*	Tan saddle on seed coat	Harosoy	1961 at Urbana
T241H	*st2*	Asynaptic sterile	S54-1714 (from same cross as Wayne)	About 1956 at Columbia, Missouri
T242H	*st3*	Asynaptic sterile	AX54-118-2-8 (Blackhawk x Harosoy)	At Lafayette, Indiana
T243	*df2*	Dwarf	Colchicine-treated Lincoln	At Ames, Iowa
T244 (Adams 77-2)	*df3*	Dwarf	Neutron-irradiated Adams	At Ames, Iowa
T249H (L67-4408A)	*(P1)*	Whitish yellow seedling, lethal	F3 (Clark^{6} x PI 84.987) x (Clark^{6} x T145)	1964 at Urbana
T250H (L67-4439)	--	Lethal seedling	F2 Harosoy^{5} x (Clark^{6} x Chief)	1964 at Urbana
T251H (L67-4440A)	*mn*	Miniature plant	F2 Harosoy^{5} x T139	1961 at Urbana

Strain	Genotype	Phenotype	Parental origin	When and where found
T 252 (L64-2612)	--	Pale green leaves	F3 Harosoy[6] x T 139	1963 at Urbana
T 253 (L67-4415A)	*y20 (k2)*	Yellowish green leaves, weak plant	T 239	1963 at Urbana
T 254 (L67-4412A)	--	Greenish yellow leaves	F2 Clark[6] x T 176	1964 at Urbana
T 255	*lf2*	7-foliolate	Hawkeye	1966 at Ames, Iowa
T 256	*df4*	Dwarf	Hark	1966 at Ames, Iowa
T 257H	*y16*	Near-lethal white	C1128[8] x Mukden (C1128 is from Wabash x Hawkeye)	At Lafayette, Indiana
T 258H (A72-1103-6)	*st4*	Desynaptic sterile	Hark	1968 at Ames, Iowa
T 259H (L71L-06-4)	*ms2*	Male sterile	F3 of SL11 (Wayne-*r Rpm Rps1)* x L66L-177 (Wayne x (Hawkeye x Lee))	1971 at Eldorado, Illinois
T 260H (N69-2774)	*ms1* (North Carolina)	Male sterile	Unknown	1966 in a farmer's field in North Carolina
T 261 (S56-26)	*k2*	Tan saddle on seed coat	Mandarin (Ottawa)	Before 1956 at Columbia, Missouri
T 262	--	"Double pod"	SRF200 (Hark-*ln)*	About 1971 at soybean Research Foundation, Mason City, Illinois
T 263 (A76-2)	*df5*	Dwarf	Harosoy 63 x PI 257.435	1968 at Iowa State University nursery, Hawaii

Strain	Genotype	Phenotype	Parental origin	When and where found
T 264 (L58-2749)	*Pd2*	Dense pubescence	Neutron-irradiated Blackhawk in the N2 generation	1956 at Urbana
T 265H (L75-0324)	*y19*	Delayed albino	F2 Williams[6] x T 259	1974-75 greenhouse at Urbana
T 266H	*ms1*(Urbana)	Male sterile, (Higher female fertility than T 260, T 267, and T 268)	F3 row of L67-533 (Clark[6] x Higan) x SRF300	1971 at Urbana
T 267H (L56-292)	*ms1*(Tonica)	Male sterile	Semisterile plant found in Harosoy	1955 by F.M. Burgess at Tonica, IL.
T 268H (A73g-21)	*ms1*(Ames 1)	Male sterile	Semisterile plant found in T 258H	1970 at Ames, Iowa
T 269H (L70-8654)	*fs1 fs2*	Structural sterile (T 269H is from *Fs1 fs1 fs2 fs2* plants)	Flower structure mutant segregating in a plant-progeny row from PI 339.868	1970 at Urbana
T 270H (A78-286)	*y22*	Greenish yellow leaves, very weak plant	Segregating in an F2-plant-progeny row from an outcross in T 271H	1977 at Ames, Iowa
T 271H	*msp*	Partial male sterile	40-parent bulk population (AP6 (s1)C1)	1975 at Ames, Iowa
T 272H (A71-44-13)	*st5*	Desynaptic sterile	Uniform Test entry W66-4108 from Merit x W49-1982-32 (W49-1982-32 is from Hawkeye x Manchu 3)	1970 at Ames, Iowa
T 273H (A72-1711)	*ms3*	Male sterile	Semisterile plant in an F3-plant-progeny from Calland x Cutler	1971 at Washington, Iowa
T 274H (A74-4646)	*ms4*	Male sterile	Semisterile plant in Rampage	1973 at Ames, Iowa
T 275 (A77-K150)	*cyt-Y2*	Yellow leaves, becoming greenish yellow	Chimeric F2 plant A75-1165-117 from T 268H x (PI 101.404B x Clark[6])	1975 at Ames, Iowa

Strain	Genotype	Phenotype	Parental origin	When and where found
T 276	*nr1*	Constitutive nitrate reductase absent	M2 generation of Williams treated with EMS, nitrosoguanadine, & X-rays	1979 at Urbana
T 277H	*ms5*	Male sterile	Semisterile plant in the M3 generation of neutron-irradiated Essex	1976 at Blacksburg, Virginia
T 278M	*cyt-Y3*	Yellow leaves, very weak plant (mutable plants are chlorophyll chimeras)	Chimeric plant of unknown source	1972 at Ames, Iowa
T 279 (D76-1609)	*lps**	Short petiole	F3 (Forrest[2] x Soden-daizu (PI 229.358)) x D71-6234. D71-6234 is a selection from a high protein Lee type x PI 95.960.	1976 at Stoneville, Mississippi
T 280 (C1640)	*fan*	Low linolenic acid	Century treated w/ mutagenic agent ethyl methanesulfonate. C1640 is the progeny of a 1981 M2 plant	1981 at West Lafayette, Indiana
T 281 (L58-617)	--	Dwarf plant, rugose leaf	Offtype in PI 232.992	1955 at Urbana
T 282 (L81-5482)	--	Curled leaf	Abnormal mutant or segregant in F3 of Williams x PI 82.278	1980 at Urbana
T 283 (A77-86)	--	Chlorophyll deficient	F7 plants of PI 101.404B x Clark[6]	1977 at Ames, Iowa
T 284H (Wabash male-sterile)	--	Male sterile (=ms3)	Outcrossed male-sterile plant in Wabash	1973 by H.K. Chaudhari & W.H. Davis
T 285 (IL3-1)	*fr5*	Nonfluorescent seedling	Williams treated with gamma rays	1981-1984 at Ames, Iowa
T 286 (MS2060)	*df6*	Dwarf	C1421 (Adelphia[8] x Mukden) treated with EMS	Early 1980's West Lafayette, Indiana

Strain	Genotype	Phenotype	Parental origin	When and where found
T 287H	*ms1*(Ames 2) (S85-62-11)	Male sterile	Segregating in $S_{4:5}$ progeny from AP 6(S1) C1	1984 at Ames Iowa
T 288	*y23* (Williams 80-7)	Leaves becoming yellow-white and necrotic, viable plant	Williams	1980 at Williams, Indiana
T 289	*Got-c* (Hardee 2)	Glutamate oxaloacetic transaminase variant	Hardee	1983 at Durham New Hampshire
T 290H	*ms1*(Danbury)	Male sterile	Beeson outcross	Prior to 1988 at Danbury Iowa
T 291H	*ms3* (Plainview)	Male sterile	F_2 (Viking x Classic II) x (Mitchell x Columbus) 'Viking' was a private line from Merit x Amsoy	Prior to 1988 at Plainview, Texas
T 292H	*ms4*(Fisher)	Male sterile	Corsoy	Prior to 1988 at Fisher, Arkansas
T293	*sp1* (Altona-*sp1*)	B-amylase null	Altona	Prior to 1978 at Durham, New Hampshire

APPLICATION FOR ENTRY INTO THE SOYBEAN GENETIC TYPE COLLECTION

Date:_____

Submitted by:_____

_____

_____

_____

_____

Return to:

R. L. Bernard, curator
Soybean Germplasm Collection
Department of Agronomy
University of Illinois
1102 S. Goodwin
Urbana, IL 61801

Genotype:_____

Phenotype:_____

(List the gene(s) and a term or description of the phenotype for the trait which is the reason for this strain being in the Genetic Type Collection.)

Parental Origin:_____

_____

_____

_____

When and where found and by whom:_____

_____

_____

(Include year, location, institution, and name of individual making find or in charge of research.)

Description: Maturity group_____ Stem termination_____

Flower color_____ Pubescence color_____ Pubescence type_____

Seed coat luster and color_____ Hilum color_____

Special instructions for growing or maintenance if any:_____

_____

Literature reference:_____

_____

_____

(List the reference(s) that first and best describe the discovery and inheritance of the trait.)

(Please send relevant reprints to the curator. List relevant manuscripts in preparation and send to curator when available.)

Seedlot received at Urbana:_____(date) T-number assigned T_____

Canada

AGRICULTURE CANADA
Research Station
Harrow, Ontario, CANADA N0R 1G0

245

1) Inheritance of an anomalous flavonol glycoside

Buttery and Buzzell (1975) proposed a scheme of genetic control of the biosynthesis of flavonol glycosides in soybean leaves with glucose or rhamnose units being added by 1-2 or 1-6 linkages to the monoglucoside under the control of the dominant alleles Fg1, Fg2, Fg3 and Fg4 to form diglycosides or triglycosides of quercetin and/or kaempferol. With thin layer chromatography (TLC) of leaf extracts, nine spots that occur on TLC plates have been identified as kaempferol glycosides and nine analogous spots as quercetin glycosides (Buttery and Buzzell, 1973). Phenotypically on TLC plates, various combinations of these spots occur as flavonol classes 1t to 16t and 1T to 16T, the former occurring when gene t is present and the latter when gene T is present (Buzzell and Buttery, 1973, 1974).

Broich (1978) observed an anomalous phenotypic pattern in 18 of 373 accessions of Glycine soja Sieb. & Zucc. tested for flavonol glycosides. The anomaly was that "Q/K7" occurred in considerable quantity in the presence of "Q/K1"; this had not been observed in G. max (Buttery and Buzzell, 1973). Subsequent genetic tests, which are presented below, have shown that segregation occurs for "Q/K1" in the presence of "Q/K7". The "Q/K1" runs very similarly to that expected for Q1 and K1; however, on the average the anomalous spots tend to be smaller than Q1 and K1. Chemical composition has not yet been determined. Since these spots are phenotypically similar to Q1 and K1 they are designated Q1B and K1B.

PI 366.123 (Maturity Group IV) was one of the accessions having the anomalous flavonol pattern. Leaf samples of F1 plants from PI 366.123 x 'Beeson', showed 72 present: 29 absent, which gave a good fit to an expected 3:1 ratio for a single gene with two alleles, dominant and recessive. All plants had Q7/K7 present. An F5-derived homozygous tt line, OX683, having K1B present was selected for further study.

The cross of OX936 x OX683 was made. The genotype of OX936 is fg1 fg2 fg3 fg4T. There is a close linkage between fg4 and T with fg3 also being in Linkage Group 1 (Buzzell and Buttery, 1974). In the F_2, segregation for spot 1B was 130 present: 41 absent which gave a good fit (P=0.8-0.7) to an expected 3:1 ratio. A TT line, OX707, was selected that had Q1B/K1B present. It was obtained as a homozygous line on the basis of 20 F_3 plants of its progeny being analyzed. The F_3 plants from five other F_2 plants segregated 123 with Q1B/K1B present: 48 with Q1B/K1B absent, which gave a good fit to a 3:1 ratio (P=0.5-0.3).

The cross of OX937 x OX707 was made. The genotype of OX937 is fg1 fg2 fg3 fg4t. In the F_2 , segregation was 51 Q1B/K1B present: 19 K1B present: 18 Q1B/K1B absent: 8 K1B absent which gave a good fit (P=0.70-0.50) for a 9:3:3:1 ratio expected for a flavonol glycoside gene segregating independently of T/t. A tt line, OX730, was selected that had K1B present. Preliminary results indicate that the additional dominant flavonol glycoside allele is not at the Fg3 or Fg4 loci. This flavonol glycoside allele is being combined in crosses with each of the other Fg genes for definitive allelism tests.

References

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Acknowledgements: S. L. Broich discovered the anomalous material and advised us of it; R. G. Palmer provided seed of the PI 366.123 x Beeson cross.

R. I. Buzzell
B. R. Buttery

China

NORTH EAST AGRICULTURAL COLLEGE
Harbin
THE PEOPLE'S REPUBLIC OF CHINA

1) Study on improving vining trait from the hybrid of G. soja X G. max with dwarf genes.

Genetic improvement of cultivated soybean may be obtained by transferring desired genes from G. soja to G. max with a backcrossing program. However, additional backcrossing usually makes the progeny contain less percentage of G. soja germplasm and most good genotypes (such as high protein content) would be lost. In our research an attempt was made not only to discard the undesired characters of G. soja (such as vining stem and black seed coat color), but also to introduce high protein genes from G. soja to cultivars as much as possible.

Materials and methods: Two groups of combinations were used in this study. One was developed from the hybridization between G. max with df gene and G. soja. The other was made by crossing G. max containing Df gene with G. soja and also by backcrossing the G. max to the F1 hybrid of G. max X G. soja (see Table 1). All parents and progenies were grown in the field of NAC Experimental Station in Harbin in 1986, and after harvesting growth period, number of branches, number of nodes in main stem, length between nodes, number of pods per plant, number of seeds per plant, 100-seed weight, oil content, foliar color and colors of seed coat were measured and evaluated. Protein content was determined by using a Tecator 1030 Analzyer. The normal distribution of traits was examined by deviating and peak degree test.

Results and discussion: Based on the results presented in Table 2, we postulated that the dwarf character of soybean was controlled by a main recessive gene. This result was in agreement with that reported by Heng-he Cheng (1985) and Kilen and Hartwig (1975). Twenty-five percent of individuals were dwarf and erect in F2 generation in dwarf variety X G. soja cross.

In the F2 population, plant height of dwarf variety X G. soja cross appeared not only as a qualitative trait but also as a quantitative trait and was modified by a few minor effective genes besides the main gene. In Figure 1, the plants were divided into tall group and short group. According to deviating and peak degree test, both of them fit to normal distribution.

Dwarf and erect traits were significantly correlated with yellowish seed coat and deep green leaves (Tables 3 and 4). Generally, the foliar green color on erect plants was deeper than that on viny ones. Therefore, it was an effective way to select more phenotypes similar to cultivated soybeans by discarding viny plants in earlier growth period. In this research, dwarf and erect traits were not correlated significantly with 100-seed weight, protein and oil content. It seemed that selecting erect individuals might not reduce seed weight and protein content

The desired phenotypes having high protein, erect stem and yellowish seed coat, produced from dwarf variety X G. soja cross, were more than 5.05%, while those from taller variety X G. soja cross with a backcross generation were only

Table 1. Parental performance of traits and cross pattern in soybeans (1986, Harbin)

Crosses	Parents	Growth period (day)	Plant height (cm)	Weight of 100-seed (g)	Protein (%)	Oil (%)	Color of seed coat	Foliar colors
A	Dwarf soybean(♀) Long 79-6330 (♂)[a]	108	42.2	22.61	43.1	19.5	yellow	deep green
B	Dwarf soybean(♀) Long 79-3407-1 (♂)	96	118.8	3.42	47.1	11.6	brown	light green
C	NAC33 (♀) Long 79-6330 (♂)	114.2	223.3	1.32	45.9	7.0	black	light green
D	NAC4 (♀) Long 79-6330 (♂)	117.8	89.4	17.8	38.7	17.2	yellow	middle green
E	NAC33 (♀) (NAC33 X Long 79-6330)F1(♂)	127	87.4	24.57	42.33	18.3	yellow	middle green
F	NAC4 (♀) (NAC4 X Long 79-6330)F1(♂)							

a. Long 79-6330 and Long 79-3407-1 are wild soybeans

0.04% to 0.9% (see Table 5). In addition, the time needed for the improvement of the progenies of dwarf variety X G. soja combination was far shorter than that spent for the improvement of taller variety X G. soja cross.

Table 2. X^2 test of dwarf and viny characters (1986, Harbin)

Crosses	Dwarf and erect ind.	Tall and viny ind.	Theoretical ratio	df	$X_c^2 < X^2_{0.05,1}$
A	28	68	24:72	1	0.68<3.84
B	53	207	65:195	1	2.74<3.84

Table 3. Correlation of dwarf trait and foliar color in soybeans. (1986 Harbin)

Crosses	Foliar color	Dwarf	Viny	Total	$X_c^2 < X^2_{0.01,1}$
A		20	11	31	
B	deep green	47	5	52	
A		8	57	65	25.22>3.84**
B	light green	6	202	208	190.9>3.84**
A		28	68	96	
B	Total	53	207	260	

** significance in 0.01 level

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Table 4. The comparison of genetic effects among the five crosses in F2 generation of soybeans. (1986, Harbin)

Crosses	Growth period (day)		Length between nodes (cm)		Weight of 100 seed (g)		Protein (%)		Oil (%)		Proportion of desired indi. (%)
	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	$\bar{X}$	CV	
A	120.1	7.3	4.0	33.1	5.28	23.6	48.1	5.9	11.3	15.2	5.05
C	123.6*	8.0	6.2*	14.8	5.8	24.1	44.9**	4.8	12.4	12.4	0
E	119.9	7.3	5.5*	16.3	14.9**	16.3	43.7**	4.9	15.4**	8.4	0.98
D	121.8	8.5	6.4*	21.2	4.9	19.8	45.6	5.72	11.2	13.2	0
F	123.4*	10.71	5.5*	15.9	8.2*	27.6	41.7**	5.5	14.5**	9.3	0.04

** significance at 0.01 level
* significance at 0.05 level

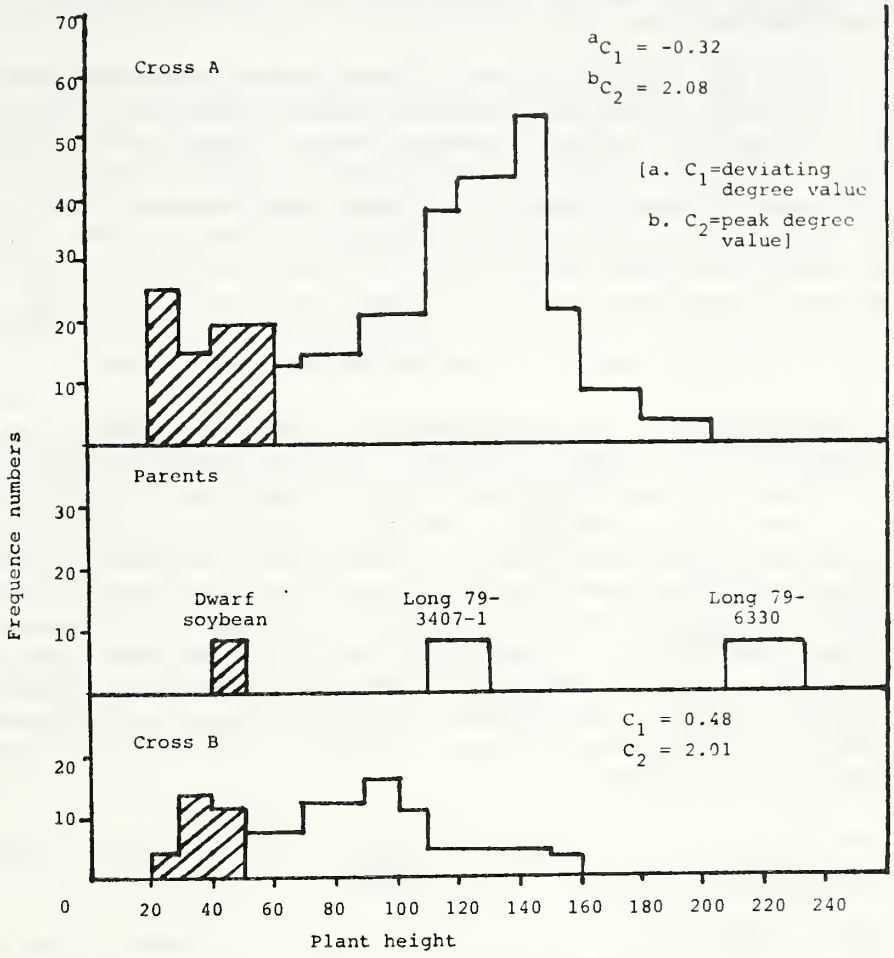

Figure 1. The frequency distribution of plant height in the F2 generation.

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2) An analysis of diallel crosses between the lines of interspecific hybrids and cultivars in soybean. I. Overcoming small seed trait of wild and semi-wild soybeans.

In recent years the introgression of wild germplasm for soybean improvement has been especially important, and some lines with high protein content have already been developed by hybridization among G. max, G. gracilis and G. soja. However, the deleterious trait of small seed size from wild relatives was also transferred to those lines along with desired traits because of linkage or pleiotropy. Based on this background, the present research was undertaken to investigate the possibility for combining the high protein trait of wild and semi-wild soybeans with large seed trait of cultivated varieties by means of 4 X 4 partial diallel crosses between the lines of interspecific hybrids and the cultivated varieties having various seed sizes.

Materials and methods: The experiment was conducted during the summer of 1986 at NAC Experimental Station in Harbin. Sixteen matings were made by crossing each of the four cultivars HAI26, NAC4, SAI4 and XHD with four interspecific hybrid lines Wild-I, Wild-II, Semi-I and Semi-II (see Table 1). The F1 generation of each cross was grown in a randomized complete block design with two replications. Each entry consisted of a single 3-meter row. Plant-to-plant distance was maintained at 10 cm. The parents and F2 population were also grown in the same field. At maturity, ten plants of each row were evaluated for growth period, plant height, number of branches, number of nodes in main stem, pods per plant, seed number per plant, seed weight per plant, 100-seed weight, oil and protein content. Protein content was determined by use of a Tecator 1030 Analyser.

The combining ability and path-coefficient analysis were worked out according to the methods reported by Liu (1984).

Results and discussions: Based on the results of Table 2, the mean of 100-seed weight in F1 and F2 generations was mainly influenced by male and female parents. Combinations involving the cultivated parent with large seed size possessed the largest 100-seed weight. Therefore, it is possible to obtain large seed phenotypes by hybridizing between cultivars with 100-seed weight more than 20g and interspecific lines. In addition, the 100-seed weight in the F1 generation was significantly correlated with that in the F2 population (Fig. 1), and the broad heritability of 100-seed weight reached 82.2% in average. This seemed to indicate that 100-seed weight was mainly controlled by additive genes. The high heritability of 100-seed weight makes it possible to select individuals with large seed in earlier generations.

General combining ability (g.c.a.) effect of 100-seed weight was significantly correlated with the mean of 100-seed weight in F2 generation (see Table 3). This information could be used in choosing parents in a program of improving seed size trait of interspecific hybrids. On the contrary, special combining ability (s.c.a.) effect of 100-seed weight was not correlated with the average value of 100-seed weight in F2 generation. The result showed that s.c.a effect of 100-seed weight was not important for the above breeding purpose.

According to the results of trait correlation and path analysis, accompanying the increase of 100-seed weight in the selected population, oil content

and seed weight per plant were simultaneously enhanced, while protein content was reduced (see Table 4). For the purpose of combining large seed trait with high protein trait, cultivated varieties with large seed size as well as with certain protein content would be appropriate to be used as cultivated parents. The use of such parents could maintain higher protein content of hybrids between cultivars and interspecific lines. In our research, although 100-seed weight was negatively correlated to seed number per plant (see Table 4), the positive action of the former was more responsible for seed weight per plant than the negative action of the latter (see Table 5). For this reason, the large seed individuals in the progenies usually had higher yield per plant.

Table 1. Combination types
(1986, Harbin)

Cultivars	Interspecific hybrid lines				Cross types	100-seed weight(g)
	Wild-I (E)	Wild-II (F)	Semi-I (G)	Semi-II (H)		
HAI26(A)	AE	AF	AG	AH	middle x small	16.0
NAC4 (B)	BE	BF	BG	BH	middle x small	18.0
SAI4 (C)	CE	CF	CG	CH	large x small	21.1
XHD (D)	DE	DF	DG	DH	small x small	13.7
100-seed weight(g)	5.3	10.9	10.8	8.2		

Table 2. The inheritance of 100-seed weight for 16 crosses in F1 and F2 generations (1986, Harbin)

Generations	Parameters	Middle x Small				Middle x Small				Large x Small				Small x Small			
		AE	AF	AG	AH	BE	BF	BG	BH	CE	CF	CG	CH	DE	DF	DG	DH
F1	$\bar{X}$	9.2	11.9	13.7	13	9.6	11.9	14	13	9.4	12.5	15	14	7.8	11	13.4	13
	S±	.73	.57	.74	.9	.8	.74	.6	.7	.4	.76	.4	.7	.6	.7	.6	.8
	CV	7.9	4.8	5.4	7	8.0	6.2	3.7	6	4.0	6.3	3	5	7.9	7	4.4	6
	$\bar{X}$ (total)	11.96				12.20				12.57				11.08			
F2	$\bar{X}$	9.1	11.9	14.0	14	9.4	13.4	14	14	9.6	12.8	17	14	8.9	12	13.7	14
	S±	1.9	2.1	4.9	1.3	1.5	1.9	1.4	4	1.0	3.1	2.4	2	1.5	1	1.6	1.5
	CV	21	17.2	13.2	10	16	13.8	10	16	14	24.0	15	14	17	9	11.4	11
	$\bar{X}$ (total)	12.23				12.75				13.18				11.96			

Table 3. The analysis of general combining ability of 100-seed weight (1986, Harbin)

Parents	Parental value (G)	g.c.a. effect	g.c.a. relative effective value
A	16.0	0.0005	0.042
B	18.0	0.252	2.113
C	21.1	0.617	5.168
D	13.7	- 0.875	-7.323
E	5.3	- 2.976	-24.908
F	10.9	- 0.362	-3.034
G	10.8	2.335	19.542
H	8.2	1.004	8.4

Table 4. Correlation and path analysis between 100-seed weight and eight other characters (1986, Harbin)

Parameter	Growth period (day)	Plant height (cm)	Oil (%)	Protein (%)	Pods per plant	Seeds per plant	Yield per plant (g)	Nodes on main stem
Correlation coefficient	.42*	-.69*	.85**	-.69*	-.5*	-.46*	.41*	.35
Path-coefficient	-.18	-.23	.05	.07	-.27	-.62*	.88*	.23
Determining	.03	.05	.00	.00	.07	.38	.77	.05

**Significant at 0.01 level; *significant at 0.05 level.

Table 5. The reaction of seed number per plant and seed weight per plant to 100-seed weight (1986, Harbin)

Traits	Reacting directly	Reacting indirectly						
		Growth period (day)	Plant height (cm)	Oil (%)	Protein (%)	Pods per plant	Seeds per plant	Yield per plant (g)
Seed no. per plant	-.62	.00	-.093	-.022	.025	-.253		.514
Yield per plant	.878	-.069	.031	.017	-.017	-.052	-.363	

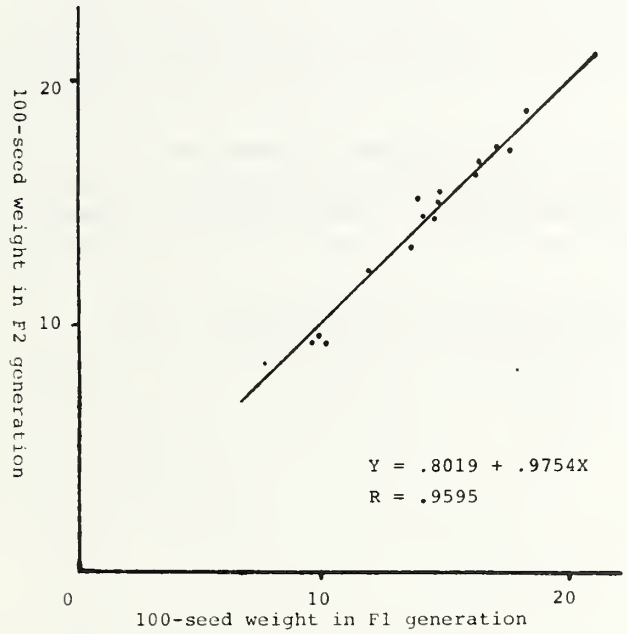

Figure 1. Correlation and regression analysis of 100-seed weight in F1 and F2 generations.

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1) Somatic cell techniques in soybean improvement: Problems, progress and potential.

Considerable progress has been made in the culture of many dicotyledonous and monocotyledonous crops, with reproducible plant regeneration from explants, callus, and protoplast-derived tissues. Protoplast fusion has already generated somatic hybrid and cybrid plants, whilst rapid advances in molecular techniques, combined with chemical and physical methods to introduce genes into protoplasts, have resulted in transgenic plants.

To some extent, many of the molecular approaches have outstripped progress at the culture level. Indeed, the fact is often overlooked that successful culture and reproducible plant regeneration are fundamental to the production of novel, genetically engineered plants. An additional complication is that most culture work remains empirical, with the response of plant material being influenced by several variables, including the physiological state of the donor material, its genotype and the composition of the culture medium.

As reviewed by Hammatt et al. (1986b) considerable progress has been made in regenerating plants from tissue cultures of legumes, but soybean presents difficulties. In particular, it is still not possible to regenerate plants from isolated protoplasts of Glycine max.

Plant regeneration from explants and callus of Glycine max: Considerable effort has been expended during the last twelve years to regenerate whole plants from cultured cells and tissues of soybean, with early studies utilizing cell suspensions. Beversdorf and Bingham (1977) induced the formation of growth centers in liquid cultures of G. max, G. soja, and G. tabacina, which produced elongate structures similar in appearance to zygotic embryos in medium with 2,4-D and kinetin. Later, Phillips and Collins (1981) produced numerous somatic embryos, particularly in G. soja. One shoot was recovered in G. soja but this did not develop into a plant. Other studies have also indicated that 2,4-D can induce embryogenesis in two accessions of G. soja and three cultivars of G. max (Gamborg et al., 1983a). The herbicide Picloram had a similar effect to 2,4-D, while inhibitors of terpene and GA3 synthesis such as AMO 1618, CCC, PP-333, and ancymidol enhanced embryogenesis. Plants were not obtained in these studies. More recently, Kerns et al. (1986) transferred hypocotyl and cotyledon callus of soybean to liquid medium containing 2,4-D, but again failed to recover plants from embryoids.

Other workers have succeeded in regenerating plants from soybean nodal explants. Cheng et al. (1980) stimulated multiple shoot bud formation from cotyledonary node segments. Germination of seeds in the presence of BAP and IBA induced bud formation at the node region; subsequent culture of node segments from these conditioned seedlings on medium with the same hormones promoted shoot bud development. Bud growth followed reduction of the BAP level, and shoots rooted and produced plants on auxin-free medium. Similar studies from the same laboratory used stem node explants from 2-week-old seedlings (Saka et al., 1980). Substituting fructose for sucrose as carbon source also promoted bud growth.

In work at the Monsanto Company, Wright et al. (1986) reported extensive shoot formation using a procedure similar to that of Cheng et al. (1980). Dissection of the cotyledonary nodes into quadrants and culture of the latter basipetally on BAP-containing medium stimulated prolific shoot regeneration. Regeneration was also influenced by explant age, and cultured nodes with intact cotyledons still attached showed decreased shoot regeneration. This method was applicable to a variety of soybeans from maturity groups 00 to VIII, with regenerated plants being grown to maturity in the glasshouse.

Leaves have been used as source material, with Wright et al. (1987) reporting plant regeneration from cultured primary leaf tissue of 27 varieties of G. max. Leaf explants (2.1 to 4.0 mm in size) from 5-day-old seedlings produced buds on medium with 2,4,5-T and adenine sulphate. Addition of BAP or Picloram and pyroglutamic acid stimulated regeneration. Interestingly, the type of culture vessel used also influenced the response, shoots developing in glass tubes but not in plastic Petri dishes. As emphasized by Wright et al. (1987) the ability to regenerate plants from leaves is significant since leaf tissues are now used routinely in the transformation of a range of plants by Agrobacterium-based vectors.

Several workers have used zygotic embryos as tissue source in the hope that such material would be more amenable to culture. Christianson et al. (1983) were the first to report a morphologically competent suspension culture derived from embryonic axes of G. max cv 'Mitchell'. These workers used a Murashige and Skoog salts-based medium containing high levels (5.0 mg l^{-1}) of 2,4-D and modified by replacement of the normal nitrogen sources with ammonium citrate. Non-friable tissue produced somatic embryos following removal of the 2,4-D from the medium combined with a change from ammonium to ammonium and nitrate. Somatic embryos transferred to the medium of Cheng et al. (1980) developed into plantlets. Subsequently, Lippmann and Lippmann (1984) induced somatic embryo development on cotyledons from immature embryos of G. max following treatment with 2,4-D or MCPA. Li et al. (1985) used callus derived from young embryos to produce a suspension from which single cells were harvested. The latter produced proembryos when cultured in hanging drops; such proembryos gave callus which itself developed globular and heart-shaped somatic embryos on medium with BAP and IAA. Plants were produced when callus was returned to liquid medium, but it was not confirmed whether these plantlets developed into mature plants.

The most comprehensive and well documented accounts of somatic embryogenesis from zygotic embryos are those of Ranch et al. (1985,1986), who employed late cotyledonary/early mature stage embryos as source material. The 2,4-D concentration in the Murashige and Skoog-based medium had a significant effect on embryogenesis, with 2,4-D at relatively high levels stimulating large numbers of embryos within two weeks of culture. The embryos produced were morphologically and structurally more normal at the two highest levels of 2,4-D, in agreement with the findings of Lazzeri et al. (1985). Inclusion of maltose or glucose as carbon source in the medium was beneficial for embryogenesis. Morphologically normal somatic embryos were morphogenetically competent, and developed into plantlets after transfer to hormone-free medium. Subsequent culture on media containing IBA gave plants that survived transfer to soil.

Although 2,4-D has been used most extensively to stimulate embryogenesis from zygotic embryos, Barwale et al. (1986) reported success using 43 µM NAA, with thiamine and nicotinic acid enhancing the response. Lazzeri et al. (1987a) further studied the effects of NAA on somatic embryogenesis, and concluded that this auxin stimulated embryogenesis more than 2,4-D with a higher proportion of somatic embryos resembling zygotic embryos. Abnormal embryos were more frequent at lower pH (Lazzeri et al., 1987b). Barwale et al. (1986) also obtained regeneration from embryo-derived callus, the procedure in this case requiring 13.3 µM BAP, 0.2 µM NAA and the concentration of Murashige and Skoog salts increased four fold. Overall, 54 genotypes showed a similar response.

More recently, Hammatt and Davey (1987) reported embryogenesis from globular, heart-stage, and cotyledonary zygotic embryos using B5 salts and vitamins, 2% sucrose, 0.1 mg l^{-1} IBA and 10% v/v coconut milk, the culture medium being made semi-solid with agarose. Heart-stage embryos were most responsive. Interestingly, germination of somatic embryos to plants was enhanced by a desiccation period.

Studies with wild Glycine species: Since wild species are important germplasm reserves, effort has also been directed towards developing regeneration systems for perennial Glycine species. Gamborg et al. (1983a) observed structures resembling early stage embryos in suspensions of G. soja and G. tabacina, while Kameya and Widholm (1981) regenerated plants from hypocotyl explants of G. canescens and G. tomentella, the shoot buds probably arising from pre-existing meristems. Later, Widholm and Rick (1983) produced shoots from callus of G. canescens, but the regenerants were difficult to root. Using the same species, Grant (1984) demonstrated that callus from embryo cotyledons underwent somatic embryogenesis. Both Hymowitz et al. (1986) and Hammatt et al. (1986) extended these studies to include G. clandestina. The former workers screened a large number of accessions from a range of Glycine species, but only obtained plants from cotyledon explants of a single genotype of G. clandestina. In the latter work, we used a B5-based medium containing 4.4 uM BAP and 0.025 µM IBA to induce callus from seedling cotyledons, leaves and petioles. Only hard, green, nodular callus produced buds, and of five accessions evaluated, two (G1231 and G1145) were morphogenetically competent. Buds developed into shoots on transfer of callus to a similar medium with reduced BAP. Shoots rooted on hormone-free medium supplemented with 0.2% activated charcoal. An extension of this work with 30 accessions showed 12 within the species G. canescens, G. falcata, G. latrobeana, and G. tomentella to be morphogenic. G. canescens G1171 was superior to the rest, with plantlet recovery from more than 70% of the cultures. In the case of G. canescens G1171, regeneration still occurred, albeit at lower frequency, when BAP was replaced by 1.0 mg l^{-1} kinetin, 2,1-P or zeatin. More recent screening has demonstrated that this regeneration procedure can, with modification, also be applied to G. argyrea (Hammatt and Davey, 1988c), and to G. cyrtoloba G1418 and G. latrobeana G1252 (Jones et al., 1988).

Plant regeneration from Glycine protoplasts: Friable callus of G. max produces rapidly growing suspensions, the cells of which release protoplasts capable of rapid division. Indeed, division of such soybean protoplasts was first reported several years ago (Kao et al., 1970, 1971; Miller et al., 1971). These protoplasts have been used extensively in fusion studies

(Mroginski and Kartha, 1985). Seedlings and mature plant organs also have been employed as source material, with callus production from pods (Zieg and Outka, 1980) and mesophyll (Gamborg et al., 1983b; Oelck et al., 1983; Tricoli et al., 1986) protoplasts, and protoplasts from cotyledons of immature seeds (Lu et al., 1983). Seedlings have been particularly useful because of their ease of production, with callus being obtained from protoplasts of root tips (Xu et al., 1982), cotyledons (Lu et al., 1983) and hypocotyls (Hammatt and Davey, 1988a).

Despite the variety of source material for protoplast isolation and the ability to stimulate plant regeneration from complex explants, there are no reports, to date, of plant regeneration from protoplasts of G. max. In contrast, considerable progress has been made with protoplasts of some wild Glycine species. Gamborg et al. (1983b) produced embryo-like structures from protoplast-derived callus of G. soja and G. tabacina, while Newell and Luu (1985) first reported shoot regeneration from hypocotyl protoplasts of G. canescens. Whilst it does not follow that accessions capable of plant regeneration from explants will also regenerate from protoplast-derived callus, it has been possible, using this basic information, to develop procedures for reproducible plant regeneration from seedling cotyledon protoplasts of G. argyrea (Hammatt and Davey, 1988c), G. canescens G1171 and G. clandestina G1231 (Hammatt et al., 1987). Enzymatically isolated protoplasts developed into cell colonies when cultured in agarose-solidified droplets of a rich Kao-based medium. Subsequent transfer of tissues to media similar to those used for explant-derived callus (Hammatt et al., 1986a) induced shoot regeneration and whole plant recovery. An important feature ensuring success of these experiments was the ability to recognize tissues capable of morphogenesis, since only hard, green, nodular callus underwent regeneration. This may be an important factor in future attempts to regenerate plants from protoplast-derived tissues of G. max.

Application of basic culture technology to soybean improvement: The ability to regenerate soybean explants and callus now enables material to be assessed for variation such as that described by Barwale and Widholm (1987), an approach which may produce novel plants of interest to the breeder. Similarly, the ability to micro-propagate perennial Glycine species from explants has application in the conservation of germplasm, since seeds of some species are difficult to obtain and available only in limited numbers (Hammatt and Davey, 1988b).

In spite of limitations imposed by the current inability to regenerate plants from protoplasts of G. max, progress is being made in applying somatic techniques to the genus Glycine. Electrofusion of seedling hypocotyl protoplasts of G. max with seedling cotyledon protoplasts of G. canescens G1171 produced heterokaryons which were cultured to callus, the latter being somatic hybrid in nature as judged by its aspartate amino transferase banding pattern (Hammatt et al., 1988). An important feature of this study was the application of high technology flow cytometry, based on differential fluorescence of the parental protoplasts, to select heterokaryon-enriched populations for culture.

Several crop species have now been transformed following direct uptake of DNA into protoplasts mediated by PEG or electroporation, or by the introduction of genes using the natural infectivity of Agrobacterium. In transformation studies with soybean, Pedersen et al. (1983) induced

crown gall tumours on G. max using wild type strains of A. tumefaciens, while Owens and Cress (1985) carried out a detailed investigation of the response of several plant genotypes to Agrobacterium strains harbouring Ti or Ri plasmids. Facciotti et al. (1985) at Calgene obtained kanamycin-resistant tissues following injection of seedling cotyledons, nodes, and internodes with an engineered strain of A. tumefaciens. The kanamycin-resistance gene was liked to a 5' portion of the soybean small subunit RuBP carboxylase gene, and a comparison of the kanamycin resistance of light-grown and dark-grown tissues demonstrated light induction of the chimaeric gene. Other work, reported from the Monsanto Company, investigated bacterial strain and plant cultivar specificity in the Agrobacterium-soybean interaction. Susceptible plant genotypes were particularly responsive to nopaline strains compared to octopine strains of A. tumefaciens and a mannopine strain of A. rhizogenes. Tissues transformed by an engineered strain of A. tumefaciens also expressed kanamycin resistance (Byrne et al., 1987). Recent work has shown that A. rhizogenes strain LBA9402 will induce transformed roots on seedlings of G. canescens and G. clandestina, and that transgenic plants containing Ri T-DNA and expressing T-DNA-specific opines can be regenerated from cultured transformed roots of G. canescens (Rech et al., 1988). Thus, the Ri plasmid may be a useful vector for future genetic engineering of Glycine species.

Basic culture techniques, combined with somatic hybridisation and transformation, have the ability to reinforce classical breeding approaches. However, considerable effort is still required in the immediate future to understand the factors governing plant regeneration from cultured soybean cells. Only when reproducible, high frequency plant regeneration is achieved will it be possible to exploit the full potential of these somatic cell techniques.

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2) Application of flow cytometry to somatic hybridisation of soybean with Glycine canescens.

We have recently emphasised the potential of protoplast fusion in facilitating gene flow from perennial Glycine species to soybean (Davey and Hammatt, 1987), and were able to demonstrate both the isolation and

culture of Glycine protoplasts, with plant regeneration from cotyledon protoplasts of G. canescens and G. clandestina.

One major obstacle faced in somatic hybridisation is the selection and culture of heterokaryons in preference to homokaryons and unfused protoplasts. Several approaches have been adopted in the past, but these rely on considerable previous research into the production of biochemical or albino mutants, or the identification of culture conditions favouring growth of hybrid material (Bravo and Evans, 1985). Soybean breeders are likely to be interested in hybrids resulting from fusion of a wide range of crop varieties with wild germplasm. Clearly, isolation of mutants or the identification of subtle culture conditions for each genotype would prove laborious, time-consuming and expensive. A more general protocol is thus required.

Green protoplasts fluoresce red due to chlorophyll autofluorescence. Additionally, Fluorescent labels such as fluorescein (green) or rhodamine (red) can be used to label protoplasts. In any fusion combination between differentially stained parental protoplasts, the heterokaryons will exhibit both fluorescent markers. Dual fluorescing heterokaryons have been isolated manually using a micromanipulator, and cultured into hybrid plants (e. g., Sundberg et al., 1987). However, this technique is laborious and time-consuming. Additionally, some hybrids have been recovered from heterokaryons isolated using flow cymetry (e. g., Afonso et al., 1985). This automated technique, whilst a relatively recent tool in plant cell biology, permits the rapid isolation of large populations of heterokaryons. Currently, we are investigating the use of this method to isolate heterokaryons between protoplasts of soybean and G. canescens G1171, for culture into hybrid plants.

Materials and methods: Protoplast isolation and culture. These protocols have been described previously (Hammatt et al., 1987; Hammatt and Davey, 1988).

Fluorescein labelling: Dark-grown soybean seedling hypocotyl protoplasts were labelled with fluorescein by the addition of 50uL of a 1.0 mg ml^{-1} acetone solution of fluorescein diacetate (FDA, Sigma) to 10-ml aliquots of enzyme solution, prior to incubation with plant tissue.

Electrofusion: Dark-grown, FDA-labelled soybean hypocotyl protoplasts, and green cotyledon protoplasts of G. canescens G1171 were washed thrice in a solution consisting of 147 mg l^{-1} $CaCl_2 . 2H_2O$ and 110 g l mannitol, and their densities were then adjusted to 1.0×10^5 ml^{-1}. Protoplasts were mixed in a 1:1 ratio. 1.0 ml aliquots of protoplasts were electrofused using an apparatus similar to that described by Watts and King (1984). Protoplasts were aligned using an AC field strength of 25-30 Vcm^{-1} (30s), and fused using a 0.2 ms DC pulse of $400Vcm^{-1}$.

Flow cytometry: Protoplasts were characterised and sorted using an EPICS V flow cytometry (Coulter) fitted with an argon ion laser operating at 457nm with 100mW power output. A 200uM nozzle was used with a drive frequency of 11.0 KHz, and CPW13M solution (Hammatt and Davey, 1988), at a pressure of 6.0 KPa, as sheath fluid. Light collected at 90° to incident laser light was filtered into wave bands of 495-465nm (fluorescein emission peak 520nm) and 630-700nm (chlorophyll peak 685nm). Subtraction was used, and heterokaryons isolated by single-droplet sorting.

Heterokaryon culture: Heterokaryons were sorted at a rate of 1000 per flat-bottomed well of a Sterilin 96 well microtitre plate, each well of which contained 30µl of liquid KP8 culture medium (Hammatt and Davey, 1988). After sorting, heterokaryons were allowed to settle, and the medium was carefully removed, in 20µl volumes, to avoid disturbance. Forty to sixty microlitres of agarose-solidified culture medium (Hammatt and Davey, 1988) were added to each well to give a final heterokaryon density of 200 per 30µl medium. The heterokaryon suspension was dispensed as 20µl-drops onto a premarked area on the bottom of a 9cm-diameter Petri dish. The remainder of the dish was filled with 30µl droplets (3ml total per dish) of G. canescens G1171 cotyledon protoplasts as nurse, also in agarose-solidified medium at a density of 6.0×10^3 ml^{-1}. After agarose gelling, the dish was filled with 10ml liquid KP8 medium, and the protoplasts cultured as described for plant regeneration (Hammatt et al., 1987).

Results and discussion: Hypocotyl protoplasts of soybean were characterized by high levels of green, but only low amounts of red fluorescence. Conversely, cotyledon protoplasts of G. canescens G1171 fluoresced primarily red, and produced minimal levels of green. Characterization of fused populations showed both unifluorescent parental protoplasts, and heterokaryons with dual red and green fluoresence.

Approx. 30-35% of heterokaryons survived flow sorting, and these preparations contained only 4-5% of parental protoplasts as contaminants. Approximately 10% of the cultured heterokaryons developed into cell colonies that survived transfer and regular subculture to semi-solid SC2 or K8 media. Early evidence from aspartate amino transferase isoenzyme analysis of heterokaryon-derived callus maintained on K8 medium, suggested that unique hybrid-specific, heteromeric proteins were produced in this tissue.

Most callus maintained on SC2 medium was green, wet and semi-nodular. After six months of regular subculture on SC2, two of the 40 heterokaryon-derived cell lines maintained on SC2 medium produced green, glossy, morphogenetic tissues. However, the shoot buds that developed were morphologically abnormal.

It is encouraging that we have been able to produce callus from flow-sorted heterokaryons between soybean and a wild relative, and to induce shoot buds from this tissue. Since preliminary analysis of callus has revealed the formation of hybrid-specific isoenzymes, it is hoped that, with further studies of factors controlling regeneration of heterokaryon-derived callus, it will be possible to recover somatic hybrid plants between Glycine species.

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3) Screening perennial Glycine species for shoot regeneration.

Recently, we have described plant regeneration in a range of perennial Glycine species (Hammatt et al., 1986; 1987a,b). Here we report results from screening accessions of G. curvata, G. cyrtoloba and G. microphylla, and additional genotypes of G. clandestina and G. latrobeana, for regeneration of shoots from explant-derived callus.

Materials and methods: Seeds were obtained from Dr. A. H. D. Brown, CSIRO Division of Plant Industry, P. O. Box 1600, Canberra, A. C. T., 2601, Australia, and sown as described previously (Hammatt et al., 1986). Leaf laminae, petioles, and leaf joints were dissected from 10-to 14-day old seedlings, and inoculated and cultured on agar SC2 medium (Hammatt et al., 1986).

Results and discussion: G. cyrtoloba: The frequency of regeneration from explants of G. cyrtoloba is summarized in Table 1.

Table 1. Evaluation of shoot production from G. cyrtoloba explants. Percentage regeneration is the total for all explant types; figures in parentheses represent explants from leaf laminae only.

	Accession Number				
	1184	1307	1267	1418[1]	1418[2]
No. explants	20	22	43	74	64
% regeneration	0	0	0	12.2(20)	35.9(40.4)

[1] Initial experiment.

[2] Repeated with regular sub-culture.

Preliminary evidence has also been obtained for shoot regeneration from explants of accession G1832.

G. clandestina: Although a single shoot developed from a leaf explant of G1308, regeneration was not observed in accessions G1868, G1869 and G1870.

G. curvata: Explant-derived tissues of accessions G1396 and G1894 did not produce shoots.

G. latrobeana: Shoot regeneration was achieved in 13% of the leaf explants tested from accession G1252.

G. microphylla: Shoot regeneration was not obtained from 50 explants of each of accessions G1428 and G1195.

Under the experimental conditions applied, G. cyrtoloba G1418 exhibited the greatest response (Table 1). These results were enhanced by regular (two weekly) sub-culture to fresh medium (Table 1), a regime which may, in the future, promote regeneration in other accessions.

The basis for different frequencies of response in accessions from the same species remains unknown. Contributing factors may include differences in media requirements, varying levels of explant-derived phenolics, and effects of the in vitro environment including conditions such as photoperiod, quality/intensity of illumination and temperature. Alternatively, the propensity to regenerate may be genetically controlled, a factor which may not be resolved by manipulation of culture conditions.

Although shoot regeneration from complex explants offers no guarantee that shoots will develop from protoplast-derived callus, some correlation has been found in the past for G. argyrea G1626 (N. Hammatt, unpublished), G. canescens G1171 and G. clandestina G1231 (Hammatt et al., 1987a). Currently, we are assessing the regenerative capacity of G. cyrtoloba G1418 protoplasts. It is hoped that such additions to the range of Glycine perennials responsive to our established regeneration procedure will prove valuable in future transformation and somatic hybridisation studies.

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4) Hairy root induction in perennial Glycine species, with regeneration of transformed plantlets in G. canescens.

Hairy root is a neoplastic disease of dicotyledons induced by Agrobacterium rhizogenes, and can be recognized as a proliferation of roots at the site of infection by the bacterium. The pathogenicity of A. rhizogenes derives from a large resident root-inducing (Ri) plasmid, a conserved part of which the T-DNA is transferred to, and stably integrated into the host genome where it is expressed. Genes on the T-DNA encode the synthesis of opines in transformed roots.

There are only a few reports, to date, of the transformation in the genus Glycine (Facciotti et al., 1985; Owens and Cress, 1985; Pedersen et al., 1983; Byrne et al., 1987), and, to date, transgenic plants have not been produced by other laboratories.

This note summarizes the susceptibility of various accessions of G. canescens and G. clandestina to infections with A. rhizogenes, and reports the establishment of hairy root cultures, with regeneration of transformed plantlets from G. canescens G1171.

Materials and methods: Bacterial strains. A. rhizogenes strain LBA 9402 was supplied by Dr. G. Oomes, Rothamsted Experimental Station, Harpenden, U. K.

Seedling growth: Seedlings of G. canescens (G1114, G1171, G1240, G1249, G1340, G1699) and G. clandestina (G1001, G1019, G1145, G1231) were produced as described previously (Hammatt et al., 1987).

Bacterial inoculation and transformed root culture: The roots were excised from 6- to 12-day-old seedlings, the hypocotyls punctured 1-3 mm below the cotyledons using a hypodermic needle, and the resulting wound sites infected with A. rhizogenes. Excised cotyledons were also inoculated both distally and proximally to the stem axis. Inoculated seedlings were transferred to hormone-free B50 medium containing B5 salts and vitamins (Gamborg et al., 1968) and 3% (w/v) sucrose. One to three weeks after infection, the hairy roots produced at the inoculation sites were excised and transferred to B50 medium supplemented with 300ug ml^{-1} of Cefotaxime (Claforan; Roussel Labs, Uxbridge, U. K.). The resulting root clones were subcultured by excising a single root tip 2-3 cm in length to fresh medium at weekly intervals. After four to five passages, the roots were transferred to, and maintained on antibiotic-free B50 medium. Cultures were incubated at 27±2°C under continuous fluorescent illumination (0.5 Wm^{-2}).

Plant regeneration: Root segments 3-4 cm in length were transferred to B50 medium containing 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA), their concentrations ranging from 1.0 to 15.0 mg l^{-1} and 0.005 to 0.05 mg 1 -1 respectively. Shoots that developed were induced to elongate on B50 medium. Shoots were maintained under similar condition to roots, but with 1.6 Wm^{-2} illumination.

Agropine test: Transformed shoots and roots were analysed for the presence of agropine as described previously (Morgan et al., 1987). The amino acid mixture used as standard contained agropine, mannopine, agropinic acid and mannopinic acid (Petit et al., 1983).

Plant DNA isolation and Southern blot analysis: Documented protocols were used for DNA isolation and Southern blotting (Morgan et al., 1987).

Results and discussion: All accessions of G. canescens and G. clandestina tested responded to infection with A. rhizogenes, the frequency ranging from 10% (G. clandestina G1001) to 70% (G. canescens G1340 and G1240). Limited callus proliferation occurred at the infection site 7 to 16 days after inoculation, with root emergence 2 to 3 days later. Cotyledon infection was less efficient than stem inoculation in both species, with cotyledon bases (proximal regions) being more responsive than tip (distal) regions.

The addition of growth regulators to the medium inhibited root growth and promoted callus formation. Shoots, which have, to date, only been obtained in G. canescens G1171, were produced from hard, green, nodular callus. Accessions unable to regenerate buds produced only undifferentiated friable callus. For accession G1171, medium containing 10 mgl^{-1} BAP and 0.05 mgl^{-1} IBA gave the highest frequency of shoot bud formation with 30% of the tissues responding. These transformed shoots, when transferred to B50 medium, developed prolific, highly branched, plagiotropic root systems.

Silver nitrate-staining compounds, which comigrated with the agropine, mannopine and mannopinic acid standards, were present in transformed roots and shoots of G1171. In contrast, tissues from non-transformed, control plantlets lacked opines. Southern blot analysis using probes for the Ri TL- and TR-DNA described by Morgan et al. (1987) confirmed integration of Ri T-DNA into the Glycine genome.

The data presented in this report demonstrate the ability to produce transgenic Glycine plants using A. rhizogenes as a vector. The Ri plasmid may constitute an efficient tool to study the expression of foreign genes in soybean tissues, and to genetically engineer the commercial crop once agronomically useful genes become available.

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1) Heterosis in Soybean

Soybean in a self-pollinated crop and the scope for exploitation of hybrid vigour will depend on the direction and magnitude of heterosis involved. Heterosis will also have a direct effect on breeding methodology in the varietal improvement. Very little work has been done on the heterotic effects of different characters in soybean. Therefore, an attempt has been made to find out the extent and nature of heterosis.

Materials and methods: Eight genetically diverse soybean varieties, 'Ankur', 'Bragg', MACS-13, 'Monetta', MACS-111, JS-2, 'Guarav' and 'Improved Pelican', promising under different agroclimatic conditions, were selected for this study. Eight parents and their hybrids were grown at the Institute's farm, Hol (Dist. Pune) in Kharif 1985 in RBD with three replications. Each treatment was sown in a single row of 5m length with a spacing of 0.45m between rows and 0.15m between plants. Observations on eight characters were recorded on five randomly selected plants of each variety and hybrid. Heterosis was calculated as percentage increase or decrease in F_1 plants over the better parent by standard procedures.

Results and discussion: Mean performance of parents for individual characters given in Table 1 suggest Improved Pelican and MACS-111 varieties to be relatively more promising.

ANOVA treatments revealed significant differences for all the characters in individual variety and their F_1 hybrids. Mean values of F_1s, expression of heterosis over better parent for different characters are given in Table 2.

Data suggest that the degree of heterosis for yield and its component characters varied considerably. Maximum positive heterosis values for individual F_1 hybrids over better parent were observed for primary branches per plant (81.75%), pods per plant (96.91%), seeds per plant (110.24%), and seed yield per plant (86.21%). Similar results of heterosis for seed yield over better parent have also been reported by other workers, 13.4% by Weber et al. (1970), 26% by Singh and Chaudhari (1974), 4.3% by Kaw and Menon (1979) and 48% by Tain (1981). However, heterosis for days to flowering, days to maturity and 100-seed weight was negligible, as also reported by Leffel and Weiss (1958). Heterosis for plant height varied from 50.14% below to 63.35% above the better parent.

Critical evaluation of the data revealed heterosis for seed yield to be due to the hybrid vigour present in the component characters which essentially depends on the relationship between seed yield and its components. The data suggest that pods and seeds per plant have contributed maximum towards yield. These findings are in conformity of earlier reports (Weber et al., 1970; Rao et al., 1978). Among the hybrids, Ankur x Monetta and Bragg x Monetta were superior over the better parent in respect of plant height and primary branches (Table 2). The F_1 hybrids, therefore, may have additive gene action for plant height and number of branches. This might have ultimately resulted in increase of total number of nodes and number of pods per plant. Thus, in these crosses heterosis for seed yield (25.11%) appeared mainly due to pods per plant (57.05%) which is cumulative

Table 1. Mean performances of soybean parents.

Sr. No.	Parents	Days to flower	Days to maturity	Plant height (cm)	No. of branches	No. of pods/ plant	100 seed wt. (g)	Yield/ plant (g)
1.	Ankur	40.75	91.05	24.61	4.08	55.58	14.99	14.10
2.	Bragg	40.00	88.33	18.83	4.00	44.33	15.71	13.77
3.	MACS-13	46.56*	103.20*	24.62	6.20	82.86	14.68	18.87
4.	I.P.	48.08*	99.77*	52.15*	7.50*	104.64*	14.28	30.22*
5.	Monetta	36.97	87.00	12.99	3.27	41.70	13.07	11.19
6.	MACS-111	47.18*	98.27*	53.90*	6.13	84.65	11.68	26.21
7.	JS-2	36.97	87.20	12.57	3.67	42.55	19.24*	14.79
8.	Gaurav	37.00	90.66	16.35	5.00	67.38	14.90	14.87
C.D. at 5%		4.44	4.68	8.32	2.19	33.16	1.31	9.70

* Significant superior at 0.05 level.

Table 2. Mean performance of F_1 and % heterosis over better parent in soybean.

Sr. no.	Crosses	Days to flower	Days to maturity	Plant height (cm)	No. of primary branches	No. of pods/ plant	No. of seeds/ plant	100-seed wt. (g)	Yield/ plant (g)
1.	Ankur x MACS-13	44.59 (-4.23)	97.77 (-5.26*)	29.06 (18.03)	7.30 (14.74)	77.08 (-6.97)	127.02 (-1.05)	14.78 (-1.40)	18.75 (0.64)
2.	Ankur x Monetta	47.95 (17.67**)	94.04 (3.28)	40.20 (63.35**)	6.44 (57.84*)	109.44 (96.91**)	197.27 (110.24**)	13.35 (-10.94*)	25.24 (79.01*)
3.	Ankur x MACS-111	45.31 (-3.96)	98.42 (0.15)	31.06 (-42.37*	7.49 (22/19)	89.95 (6.26)	154.25 (-33.29*)	14.56 (-2.87)	22.01 (-16.02)
4.	Ankur x JS-2	42.73 (4.86)	97.80 (7.41**)	25.00 (1.58)	7.20 (76.47**)	94.00 (69.13*)	123.50 (31.62)	15.89 (-17.41**)	19.69 (33.13)
5.	Ankur x Gaurav	45.77 (12.32*)	97.32 (6.88*)	32.58 (32.38)	7.35 (47.00*)	91.07 (35.16)	167.78 (68.54*)	15.17 (1.20)	27.69 (86.21**)
6.	Bragg x MACS-13	46.08 (-1.03)	97.29 (-5.73*)	33.03 (34.16*)	6.47 (4.35)	104.43 (26.03)	171.29 (33.42)	15.61 (-0.63)	26.03 (37.94)
7.	Bragg Improved Pelican	45.66 (-5.03)	95.33 (-4.45)	26.00 (-50.14**)	8.00 (6.67)	78.75 (-24.74)	136.03 (-38.10**)	14.03 (-8.15)	18.84 (-37.66*)
8.	Bragg x Monetta	44.86 (12.15*)	97.54 (10.42**)	28.97 (53.85*)	7.27 (81.75**)	77.75 (74.99*)	150.55 (76.43*)	14.78 (-5.92)	21.93 (59.26*)
Mean of parents		41.69	93.19	28.37	4.98	65.46	127.64	14.82	18.00
Mean of hybrids		45.37	96.94	30.74	7.19	102.81	153.46	14.83	22.52
Average heterosis		%8.83	4.03	8.37	44.35	57.05	20.23	0.01	25.11

Figures in parenthesis represent percentage heterosis.
*-significant at 5% level.
**-significant at 1% level.

action of primary branches and increased pod number. Finally, on the basis of seed yield, plant height, primary branches, pods and seeds per plant, the crosses Ankur x Monetta, Ankur x Guarav and Bragg x Monetta appeared to be most promising for exploiting heterosis. The data further suggest the possibility of hybrid soybean to be economical, if commercial method to produce F_1 seed is evolved.

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1) Inheritance of leaflet shape in soybean.

Leaflet shape of soybean is known to be controlled by a single gene (ln), and the broad shape is dominant to the narrow shape (Bernard and Weiss, 1973).

Takahashi and Fukuyama (1919) reported that the F1 plants resulting from a cross between broad and narrow leaf types were intermediate, while the F2 population segregated in the ratio of 1:2:1 of broad, intermediate, and narrow, respectively. However, since then many workers have shown that in soybean the broad leaf character is completely dominant to the narrow leaf character (Takahashi, 1934; Woodworth, 1935; Domingo, 1945). But several years ago at the Tokachi Agric. Exp. Stn. Hokkaido, Japan a strain 'Toiku 187' having intermediate leaflets has been true-bred from progenies of a cross between 'Suzuhime' (narrow) and 'Kitamusume' (broad).

The objective of this study was to reexamine the inheritance of the leaflet shape in soybean.

Materials and methods: Two cultivars, 'Kitakomachi' (broad leaf), 'Isuzu' (narrow leaf) and one line Toiku 187 (intermediate leaf) and their F1 and F2 progenies were used in this study. Leaf shape is classified by using the leaf shape index (LSI), which is defined as a ratio of the length to the maximum width of a leaflet. In Japan, cultivars having LSI values of<1.9 are classified as broad, between 1.9 and 2.1 as intermediate and >2.1 as narrow. In this study, LSI was determined by using the central leaflets at the second leaf from the top of the main stem. Leaflet shape was classified into two groups with LSI of <2.6 as broad and >2.6 as narrow on the assumption that the leaflet shape is controlled by a single major gene with the broad character completely dominant to the narrow character. All measurements were made on field-grown soybeans.

Results and discussion: Table 1 shows the distribution and means of LSI's of the parents, F1 and F2 progenies. The F2 populations were mixed with the reciprocal crosses, since no differences were found in the distribution of LSI's between the reciprocal crosses.

The LSI of Toiku 187 varied from 1.7 to 2.5 with a mean of 2.06 which was slightly larger than that of Kitakomachi (1.86), while the LSI for Isuzu ranged from 2.8 to 4.8 with a mean of 3.46 which was the highest among the three parents. According to the normal classification, Kitakomachi, Toiku 187, and Isuzu fall into broad, intermediate, and narrow group, respectively. However, on the basis of the new classification proposed in this study, both Kitakomachi and Toiku 187 can be grouped under the broad leaf category (LSI >2.6), while Isuzu falls into the narrow group (LSI > 2.6).

The frequency distributions of the LSI are shown in Figure 1. For the cross Kitakomachi (KK) + Isuzu (IS), a binominal distribution was obtained with 243 plants in the broad group (LSI < 2.6) and 66 plants in the narrow group (LSI > 2.6). This segregation was a good fit to the 3:1 ratio

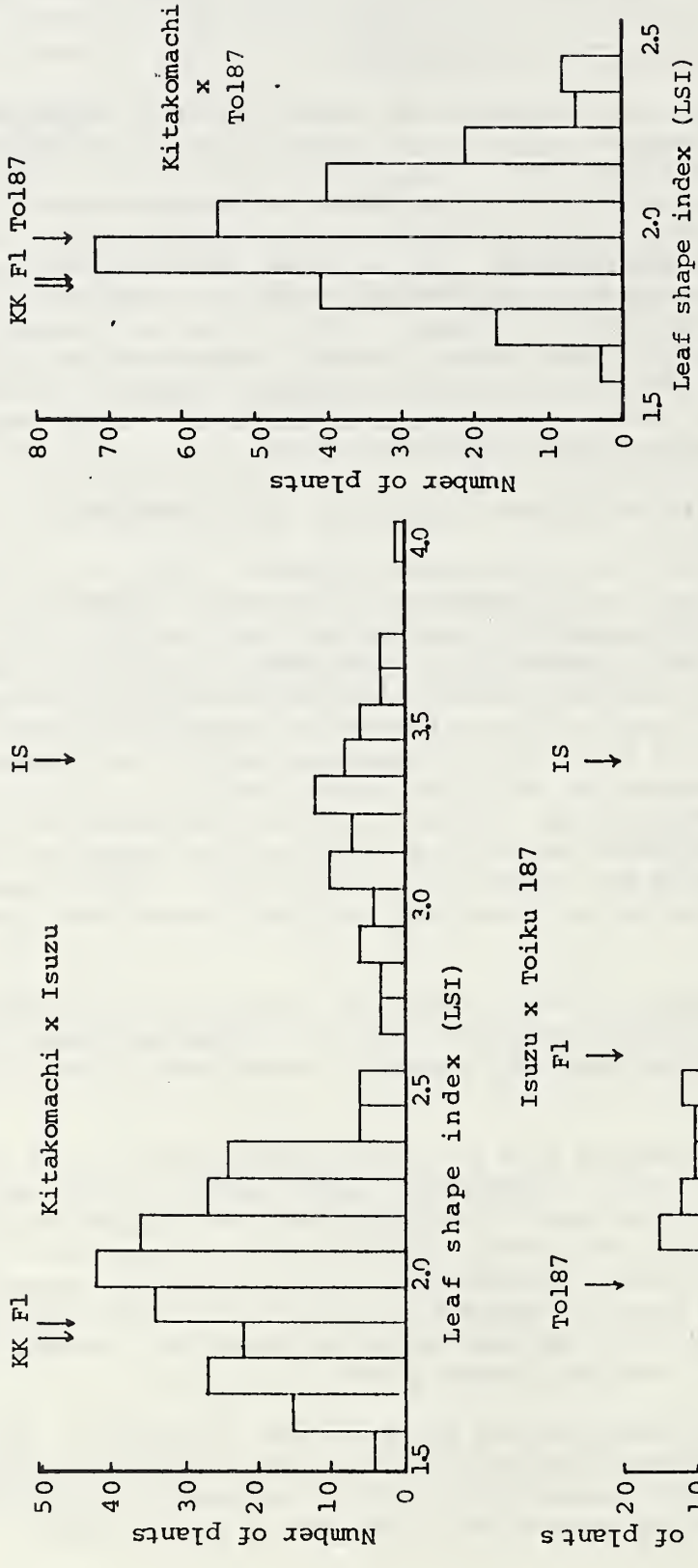

Fig. 1. Distribution of LSI in F2 of the three crosses among Kitakomachi(KK), Isuzu(IS) and Toiku 187(To187).

(X^2 value = 2.18, $0.10 < P < 0.25$). The mean LSI of F1 plants was 1.90 which is almost the same as that of the parent Kitakomachi (1.86). This suggests that the leaflet shape is controlled by a single gene and the broad leaflet shape of Kitakomachi is completely dominant to the narrow leaflet shape of Isuzu.

A normal distribution in the F2 was observed for LSI in the cross Kitakomachi x Toiku 187 (Fig. 1). The mean LSI of Kitakomachi, Toiku 187, F1 and F2 were 1.86, 1.88, 2.06 and 1.97, respectively (Table 1). In the F2 the LSI ranged from 1.6 to 2.4. According to the proposed classification, both parents, the F1 and F2 plants fell into the broad group. However, the differences in the means of Kitakomachi and Toiku 187 could be attributed to the effect of polygenes.

The F2 distribution of LSI's in the cross Isuzu x Toiku 187 was rather binominal with a wide range of 1.6 to 4.5. The mean LSI of the F1 was 2.65, which was almost intermediate between both parents. Eighty-four plants fell into the broad group while 41 plants were included in the narrow groups. This segregation is a poor fit to the 3 : 1 ratio (X^2 value = 4.01, $P < 0.05$).

Takahashi and Fukuyama (1919) used a similar index to that defined in this study. They determined the LSI's of the parents and F1 by averaging the LSI values of the central leaflets of all leaves on the main stem. The mean LSI values of the broad, narrow parents, and the F1 were 1.84, 280, and 2.12, respectively. However, they classified the F2 plants into broad, intermediate, and narrow, based only on visual observations. The leaves of F1 plants were shorter in length than that of both parents and intermediate in width.

The results obtained in this study suggested that the leaflets having LSI of < 2.6 could be classified into broad group. If this is valid, the intermediate type reported by Takahashi and Fukuyama falls into the broad group and even in their study the broad character was completely dominant to the narrow leaf character.

From the results it can be seen that leaf character is controlled by a single major gene as the cross Kitakomachi x Isuzu segregated into a perfect F2 ratio of 3 : 1. However, further studies are necessary to completely understand the behavior observed in the cross Toiku 187 x Isuzu.

Table 1. Distribution of leaf shape index of parents, and their F1 and F2 populations.

Leaf shape index	Parents KK (1)	IS (2)	To187 (3)	F1 cross of (1)x(2)	(1)x(3)	(2)x(3)	F2 cross of (1)x(2)	(1)x(3)	(2)x(3)
1.5							4		
1.6							15	3	2
1.7	1		2				27	17	2
1.8	4		6	4	2		22	41	4
1.9	3		11	2	2		34	72	5
2.0	2		26	4	1		42	55	8
2.1			22				36	40	15
2.2			12				27	21	12
2.3			6				24	6	10
2.4			4			1	6	8	10
2.5			1				6		12
2.6						1			4
2.7						1	3		4
2.8		1					3		4
2.9						1	6		1
3.0							4		2
3.1		1					10		1
3.2							7		4
3.3		3					12		
3.4		1					8		3
3.5							6		7
3.6		1					3		1
3.7							3		4
3.8		1							3
3.9									2
4.0		2					1		1
4.1									1
4.2									
4.3									1
4.4									
4.5									2
n	10	10	90	10	5	4	309	263	125
Mean	1.86	3.46	2.06	1.90	1.88	2.65	-	1.97	-

KK = Kitakomachi (broad leaf), IS = Isuzu (narrow leaf),
To187 = Toiku 187 (intermediate leaf).

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1) Induced variability studies in soybean.

M_2 seed of four soybean varieties irradiated with 500, 1000, 1500 and 2000 rads Nf were grown to study variability of seven quantitative traits: plant height, branches per plant, pods per plant, pod length, grains per pod, 100-grain weight and grain yield per plant. Positive and negative shifts in the mean values were observed in treated population. However, the coefficient of variability increased in all the treatments for the attributes under study. Combination of high heritability with high genetic advance was also recorded. This induced variability would provide an opportunity for improvement of soybean in early generations.

The low yield potential, susceptibility to diseases and insects, narrow adaptability, photoperiod sensitivity and poor seed quality of presently grown cultivars are the major constraints of soybean production in Pakistan.

Variability is prerequisite for any successful plant breeding program. Soybean being highly self-fertilized crop has very narrow genetic base. Hence the mutation breeding in soybean was initiated at AEARC, Tandojam to induce genetic variability for selecting mutants with better plant type suited to a wide range of agroclimatic conditions with high yield potential. Induced mutation breeding in the recent past has been successfully used for the improvement of various crop plants (Micke et al., 1985). Increased variability in M_2 population has earlier been reported by various workers (Mujeeb 1973, Rajput 1974 and Zakri et al.,1981). The present report discusses the effect of Nf radiation on various morphological characters of soybean in the M_2 generation.

Materials and methods: M_2 population derived from four leading soybean varieties, viz. Columbus, Loppa, Improved Pelican and T-15, treated with 500, 1000, 1500, and 2000 Rads of Nf, was grown along with respective parents. From seedling stage till maturity, the crop was thoroughly screened for various desirable attributes and quite a few selections were made for further screening and evaluation during subsequent generations. For this report at maturity 20 plants were taken in M_2 generation at random from each treatment along with parents. Data were recorded for plant height, branches per plant, pod length, pods per plant, number of grains per pod, 100 grain weight and grain yield per plant. Data were subjected to statistical analysis. The heritability was worked out according to the formula used by Shakoor et al., (1977).

$$h^2 = \frac{VM_2 - VP}{VM_2} \times 100$$

Where h^2 = heritability in broad sense

VP = Variance of respective parent

VM_2 = Variance of M_2 population

Genetic advance was calculated according to Allard (1960).

G.A = i x Standard deviation of M_2XH

Where i = Constant that reflects the selection intensity.

H = Heritability coefficient i.e. the ratio obtained by dividing the genotypic variance by the phenotypic variance.

The value for i = 1.7 in this study is based on 10% selection intensity.

Results: Genetic parameters like coefficient of variation, heritability and genetic advance expressed as percent of mean are provided in Tables 1-3.

The plant height generally decreased in all the varieties after irradiation except in Improved Pelican where improvement in height was noted. The coefficient of variability was enlarged in the treated populations as compared to their respective control. The heritability ranged from 17 to 96% in the treated populations. In Improved Pelican and Loppa maximum heritability was observed (Table 1). The values for genetic advance ranged from 3 to 45.

The branches per plant showed negative and positive shifts in irradiated populations. The coefficient of variability was generally higher in treated material. The heritability was sufficiently higher (Table 1). In some cases high heritability values were in combination with genetic advance (Columbus, 1500 rads; Improved Pelican, 2000 rads; T-15, 500 rads and Loppa 2000 rads).

In almost all the varieties treated material had more number of pods per plant as compared to their respective control. The coefficient of variability ranged from 32 to 65% as compared to 27 to 39% of control (Table 1). The heritability values ranged from 2.8 - 95.5% and genetic advance 1.9 to 75.5. Maximum heritability associated with maximum genetic advance was observed in variety Improved Pelican after 500 rad treatment.

Pod length showed quite erratic trend, in some treatments pod length increased while in others decreased. Coefficient of variation was higher in all the treated populations. The heritability ranged from 13-77% and genetic advance estimates were 2-14 (Table 2).

Grains per pod have shown both positive and negative shifts in mean values in irradiated material. However, sufficient coefficient of variation was generated (Table 2). Heritability and genetic advance estimates in variety Improved Pelican and at 1000 rads in Loppa and after 500 rads in variety T-15 were reasonably higher.

Very little variation was observed in treated and control populations for seed index (100 grain weight). Heritability values ranged from 6 to 96% and that of genetic advance 2-26 (Table 3).

Grain yield per plant like pods per plant showed great variation in the irradiated populations. Heritability ranged from 6 to 96% (Table 3). Population generated after 500 rads and 2000 rads treatments in variety Improved Pelican showed maximum heritability (96.2%) accompanied with maximum value of genetic advance (64.9).

Discussion: Yield is a complex character. In legumes the primary yield components include pods per plant, number of grains per pod and test grain weight. In all the breeding programs irrespective of the crop, yield improvement being the primary objective, vigorous selection is practiced for higher yield. Considering the observed behaviour of the mean in the treated populations of all the varieties in the present study it appears that the direction of shift in the mean for yield need not make breeders sceptical about the usefulness of mutation breeding for yield improvement. Mutants which transgress the upper range of the parent populations, though they occur at very low frequency can still provide the necessary base for the desired direction of selection. Coefficient of variability in the treated populations of all the varieties was substantially increased. This enlarged variability observed in treated population suggest that selection could be made for plants with improved yield.

Heritability and genetic advance are the most important parameters in the selection breeding program (Johnson et al., 1955). The chances of fixing and improving the particular character in a short period are great, if the heritability is high Singh et al. (1978). In the present study, high heritability estimates accompanied with high genetic advance were observed for pods per plant (Improved Pelican at 500 and 2000 rads and in variety T-15 at 1500 rads), grains per pod (Loppa at 1000 rads and in variety Improved Pelican at 1000, 1500 and 2000 rads) and grain yield per plant (Improved Pelican at 500 and 2000 rads and in variety T-15 at 500 and 1500 rads treatment). Combinations of high heritability with high genetic advance indicate that these characters are controlled by additive type of genes. This increase in heritability and genetic advance afore mentioned characters at particular treatments may be due to increased mutations and recombinations induced by mutagenic treatment. Hence by selection for these characters in the early generation from these treatments a potential gain could possibly be achieved. Variability induced in mutation breeding programs can thus be usefully exploited for removing the depressing factors involved in the expression of yield in soybean.

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Table 1. Variability, heritability and genetic advance as affected by Neutron fast irradiation in soybean

Variety/Treatment	Plant height				Branches per plant				Pods per plant			
	$\bar{X}$(cm)	CV	h^2	G.A	$\bar{X}$	CV	h^2	G.A	$\bar{X}$	CV	h^2	G.A
Columbus (Cont.)	61.3	7.7	-	-	3.7	31.9	-	-	145.5	27.0	-	-
500 rads	60.4	10.3	43.1	7.6	3.0	36.2	51.1	31.2	188.9	32.1	57.9	31.5
1000 rads	55.5	10.3	83.8	11.2	3.1	37.4	43.1	27.2	158.5	33.4	44.6	25.3
1500 rads	59.2	10.9	46.9	8.7	3.1	42.9	58.2	42.2	140.9	39.7	50.6	30.9
2000 rads	52.7	9.9	19.5	3.3	3.2	35.9	42.1	25.4	144.1	32.9	31.2	17.4
Loppa (Cont.)	102.2	7.7	-	-	3.8	15.8	-	-	144.7	35.8	-	-
500 rads	83.8	14.3	56.8	13.8	3.9	17.7	22.1	6.5	146.3	38.8	16.6	10.9
1000 rads	78.8	16.6	63.7	18.0	3.7	19.7	32.4	10.8	126.1	41.7	2.8	1.9
1500 rads	76.1	14.5	48.4	11.9	3.9	17.4	19.8	5.7	187.5	45.6	63.3	49.1
2000 rads	87.7	19.1	77.8	25.3	4.4	31.2	81.1	43.0	225.6	41.8	69.9	49.7
Improved Pelican (cont)	97.0	7.2	-	-	2.6	46.0	-	-	76.7	31.2	-	-
500 rads	128.8	27.5	96.1	44.9	3.4	51.3	50.5	43.9	242.9	46.5	95.5	75.5
1000 rads	98.3	14.6	76.3	18.9	3.3	38.8	77.7	4.9	95.3	36.7	53.4	33.3
1500 rads	104.9	12.6	71.9	15.3	3.4	38.4	12.0	7.7	102.5	38.2	62.8	41.8
2000 rads	106.7	25.2	93.3	39.9	3.4	53.4	54.3	49.3	121.0	43.5	79.4	58.8
T-15 (Cont)	74.2	7.6	-	-	4.0	24.3	-	-	105.3	39.5	-	-
500 rads	73.2	11.3	53.2	10.2	4.3	41.4	70.3	49.3	90.9	64.5	49.7	54.5
1000 rads	73.5	8.8	23.6	3.5	4.2	23.6	20.3	8.0	96.3	46.0	12.1	9.4
1500 rads	64.5	10.0	24.3	4.1	3.6	35.3	44.7	26.7	129.7	51.5	61.2	53.5
2000 rads	66.7	9.3	17.0	2.7	3.6	28.9	13.0	6.1	110.7	44.1	27.5	20.6

CV = Coefficient of variability h^2 = Heritability GA = Genetic Advance as percent of mean

Table 2. Variability, heritability and genetic advance as affected by Neutron fast irradiation in soybean

Variety/Treatment	Pod length				Grains per pod			
	$\bar{X}$ (cm)	CV	h^2	G.A	$\bar{X}$	CV	h^2	G.A
Columbus (Cont.)	4.4	3.9	-	-	2.3	13.2	-	-
500 rads	4.4	5.0	40.3	3.4	2.3	14.7	28.4	6.9
1000 rads	4.6	5.6	57.2	5.4	2.6	14.1	30.6	7.1
1500 rads	4.5	6.0	60.4	6.0	2.6	13.5	26.5	5.8
2000 rads	4.5	6.0	60.4	6.0	2.5	14.5	30.6	7.2
Loppa (Cont.)	4.0	3.3	-	-	2.1	1.3	-	-
500 rads	4.0	4.8	53.2	4.3	2.1	2.1	57.8	18.8
1000 rads	4.0	6.3	13.0	7.8	2.0	38.0	88.3	57.0
1500 rads	4.0	3.8	24.9	1.5	2.0	16.2	37.9	10.3
2000 rads	4.0	4.0	34.0	2.2	2.1	18.6	55.6	17.1
Improved pelican (cont.)	4.0	4.8	-	-	2.1	19.9	-	-
500 rads	3.9	10.4	77.4	13.5	2.3	35.9	75.0	45.7
1000 rads	4.0	8.4	66.9	9.4	2.3	39.2	77.7	51.5
1500 rads	3.8	8.9	68.8	10.2	2.1	51.9	84.5	74.3
2000 rads	3.9	8.1	64.7	8.9	2.1	44.3	79.6	59.5
T-15 (cont.)	3.8	3.1	-	-	2.1	10.4	-	-
500 rads	2.0	11.3	72.8	14.0	2.2	10.6	8.5	0.7
1000 rads	3.9	5.2	64.0	5.7	1.9	22.5	72.5	27.3
1500 rads	3.8	3.9	36.0	2.3	2.1	13.0	33.6	7.2
2000 rads	3.9	3.6	26.5	1.5	2.1	14.1	42.4	9.7

CV = Coefficient of variability h^2 = Heritability GA = Genetic Advance as percent of mean

Table 3. Variability, heritability and genetic advance as affected by Neutron fast irradiation in soybean

Variety/Treatment	100 grain weight				Grain yield per plant			
	$\bar{X}$(gm)	CV	h^2	G.A	$\bar{X}$(gm)	CV	h^2	G.A
Columbus (Cont.)	17.3	5.4	-	-	58.8	29.9	-	-
500 rads	17.6	9.9	79.8	11.9	73.9	44.8	31.1	15.1
1000 rads	18.2	10.5	75.5	13.4	53.7	36.6	20.1	12.5
1500 rads	17.5	16.8	89.7	25.5	54.1	36.5	21.2	13.2
2000 rads	18.1	10.3	74.5	13.0	58.0	32.1	11.3	6.1
Loppa (Cont.)	13.5	6.7	-	-	45.1	39.9	-	-
500 rads	14.4	6.5	6.3	0.7	46.4	40.0	6.0	4.1
1000 rads	13.7	10.8	62.2	11.4	44.5	46.4	24.0	19.8
1500 rads	13.3	13.7	75.0	17.5	50.9	51.4	52.8	46.1
2000 rads	13.8	13.7	76.9	17.8	57.4	42.0	44.3	31.5
Improved pelican(Cont.)	10.2	1.6	-	-	14.8	27.4	-	-
500 rads	11.5	10.4	1.7	0.3	49.0	42.8	96.2	64.9
1000 rads	10.4	15.8	48.2	12.9	16.1	38.7	57.2	37.6
1500 rads	11.0	14.0	42.0	10.0	19.1	29.9	49.7	25.3
2000 rads	11.4	11.0	10.9	2.0	25.9	38.9	83.7	55.3
T-15 (Cont.)	13.6	20.0	-	-	27.2	38.8	-	-
500 rads	13.7	10.6	57.1	10.2	23.2	56.1	34.4	32.7
1000 rads	13.9	12.8	71.8	15.6	24.0	48.4	17.7	14.6
1500 rads	13.3	12.4	66.9	14.1	29.7	42.4	29.5	21.2
2000 rads	13.7	8.4	31.8	4.5	25.6	49.0	29.4	24.4

CV = Coefficient of variability h^2 = Heritability GA = Genetic advance as percent of mean

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1) Shattering rate and yield losses in some soybean varieties in Cukurova region of Turkey.

Double-cropped soybean production started in 1980 in Turkey. The soybean planting area increased very fast and reached 95,000 hectares in 1986. More than 80% of the total planted area was double-cropped (Tarimsal Yap: va uretim, 1986). The Cukurova region is very suitable for double-cropped soybean production. It is located in 37°19' N latitute and covers Adana, Hatay, and Icel provinces. Cukurova region has the most productive land of Turkey. In this region, soybeans are planted after wheat harvesting. Wheat is harvested from the end of May to mid-June. After wheat harvesting, soybeans can be grown successfully as a second crop in the Cukurova region. More than 90% of the total soybean production of Turkey was produced in this region.

Double-cropped soybean is planted in June and harvested in October, depending on variety. When the soybean planting is delayed to July, it will be harvested in November. In this region it usually rains once or twice in October. For this reason, if the harvest is delayed, soybean pods will start shattering in a short time after normal warm weather returns. Thus, the yield losses will increase. Shattering rate in soybean pods depend on genotype and environmental conditions. Some of the varieties are resistant to shattering but others are susceptible (Arioglu, 1987). The objective of this study was to investigate the shattering rate and harvest losses of some soybean varieties that can be grown as a second crop in the Cukurova region.

Material and methods: This study was conducted as a second crop after wheat harvest in experimental area of the University of Cukurova in 1986. In this research, 17 soybean varieties (A3127, Washington V, Mitchell, Mitchell 410, A2943, Calland, Essex, L4106, Corsoy 79, Amsoy 71, Amcor, A2575, Lincoln, Shawnnee II, L4207, BSR-201 and L4204) were used. The experimental design was randomized block with three replications.

The maximum air temperature increased up to 39.5°C during the growing period in Adana in 1986. At the growing period the weather was dry; sufficient water was supplied by irrigation. The climate data over the growing period in Adana in 1986 are given in Table 1.

Table 1. Climatical data over the growing period in Adana in 1986

Months	Temperature (°C)			R. H (%)	Total rainfall (mm)
	Min.	Max.	Average		
June	20.4	31.4	25.3	66.7	13.8
July	21.9	37.6	27.9	72.6	----
August	23.4	36.3	25.8	72.6	----
September	17.7	39.5	27.0	64.6	3.6
October	13.7	34.0	21.9	60.0	60.3

The soil type of the experimental area is sandy-loam and soil pH ranged between 7.3-7.5. The soil organic matter is low.

The seedbed was prepared with disc harrow after wheat harvesting. Diammonium phosphate (250 kg ha^{-1}) fertilizer was broadcasted at the time of soil preparation to the experimental area. The plot sizes were 2.8X5.0 =14.0 m^2 and row spacing was 0.7 m. The soybean seeds were inoculated with Rhizobium japonicum and planted by hand in the third week of June. The seeding rate was 30 plants per m. row. After planting, all plots were irrigated to obtain a uniform emergence of stand. The experimental area was furrowed twice during the plant development to control weeds. Irrigation was applied by flood irrigation system four times. Two rows of the plots were harvested by hand at different times. For seed yield, when the varieties reached to maturity they were threshed by a stationary plot thresher. The other two rows of the plots were harvested after 20 days at the normal harvesting time, to investigate the shattering rate. The weather was rainy 5 days before the second harvesting time.

INTSOY methods were used to obtain data (Jackobs et al., 1984). The investigated characteristics are:

Growing period (days): Days from date of emergence to date when 95% of the pods were ripe.

Pod number (pod/plant): Mean number of pods per plant estimated from 20 plants.

Seed yield (kg/ha): Weight in grams in clean, dry grain from 5.0 m of two rows, which is a harvested area of 7.0 m^2. Yield per hectare estimated from plots yield in kilograms.

Shattering rate (%): 20 plants were randomly selected after 20 days of the normal harvesting time. Shattered pods were counted on each plant; shattering rate was estimated.

Yield losses (kg/ha): This parameter was calculated by multiplying normal seed yield and shattering rate.

Results and discussion: The data belonging to growing period, seed yield, pod number, shattering rate and yield losses are given in Table 2.

As can be seen in Table 2 the average seed yield per hectare was between 2376.2 kg and 3980.9 kg. Seed yield was highest in A3127 and Washington V soybean varieties.

The shattering rate varied between 2.3% and 23.5% in some tested soybean varieties. The highest shattering rate was in L4107 and BSR-201 varieties. These varieties are very susceptible to shattering. Also Lincoln, Mitchell, Washington V, and A2575 varieties' shattering rates were higher than 10%. Essex, Amcor, A2943 and Mitchell 410 varieties were resistant to shattering. These varieties' shattering rate was less than 5%.

The yield loss per hectare was estimated between 607.9 kg and 77.5 in soybean varieties. Seed loss was highest in Mitchell and Washington V varieties.

Mitchell and Washington V varieties' yield and shattering rate were very high. If we plant these varieties as a second crop in the Cukurova region, the seed yield will increase but, due to high shattering rate, the seed losses will be increased. For this reason, Washington V and Mitchell varieties must be harvested at maturity. If the farmers want to grow soybeans in a large area, they have to plant at least 50% shattering-resistant variety such as Mitchell 410 and A2943. These findings are in agreement with the results of Jackobs et al., 1983, 1984; Atakisi and Arioglu, 1983; Arioglu et al., 1986.

Table 2. Relationship between shattering rate and yield losses in some soybean varieties

Varieties	Growing period (days)	Seed yield (Kg/ha)	Pod number (Num./Plant)	Shattering Rate (%)	Yield losses (kg/ha)
A3127	103	3980.9 A	31.0 BC	8.2 GH	325.9 CD
Washington V	102	3633.3 AB	40.0 ABC	16.4 BCD	594.1 A
Mitchell 410	110	3538.1 BC	29.0 C	4.3 I	154.8 FG
Mitchell	110	3485.7 BCD	31.4 BC	17.3 BC	607.6 A
A2943	100	3395.2 BCDE	28.0 C	2.3 I	77.5 G
Calland	105	3304.7 BCDE	38.8 ABC	9.9 FG	327.6 CD
Essex	121	3266.6 CDE	41.5 AB	3.6 I	117.6 G
L4106	110	3184.4 DEF	44.3 AB	8.8 GH	278.9 CDE
Corsoy 79	99	3071.4 EFG	38.8 ABC	5.4 HI	167.4 EFG
Amsoy 71	100	2885.7 FGH	29.4 BC	9.5 G	249.5 DEF
Amcor	106	2779.6 GHI	38.9 ABC	3.1 I	86.9 G
A2575	95	2707.1 HIJ	29.8 BC	13.4 DEF	363.5 C
Lincoln	97	2704.7 HIJ	36.4 ABC	17.7 B	476.2 B
Shawnee II	102	2667.7 HIJ	45.9 A	10.7 EFG	290.4 CD
L4207	102	2623.8 HIJ	29.8 BC	23.5 A	533.5 AB
BSR-201	95	2509.4 IJ	30.3 BC	22.4 A	557.4 AB
L4204	102	2376.2 ABC	36.3 ABC	13.9 CDE	330.0 CD
CV (%)		6.9	20.8	19.4	20.6
L.S.D. (5%)		351.5	12.2	3.9	111.8

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2) Influence of Mixtalol application at different nitrogen level on soybean yield and plant characteristics.

Soybean is a very important crop for Turkey's agriculture. The soybean planting area was 3,000 hectares in 1980, then in 1986, it increased to 95,000 hectares. The reason for this increase is that soybean can be grown successfully as a second crop after wheat harvesting (Tarımsal Yapı ve üretim. 1986).

There have been very important developments in soybean agriculture in Turkey. While maximum soybean yield was 1.0-1.5 ton ha^{-1} in 1970, it reached to 4.0-4.5 ton ha^{-1} in 1986. Introduction of the new high yielding varieties and new techniques affected the yield increases (Arioglu, 1987).

More than 80% of the total soybean production of Turkey was produced as a second crop in the Cukurova region. Cukurova's environmental conditions are suitable for soybean growth and development. The high temperatures occurring in some years negatively affected the yield (Arioglu and Ersoy, 1987).

The high and low temperatures affect photosynthesis in soybean. Also, some chemicals affect the yield. The yield increases occurred with application of some chemicals such as Morphactin, Tiba, Amo 1618, GA^3, Ethephon and CCC (Tanner and Ahmed, 1974; Wilcox, 1974; Clapp, 1975; Stutte and Davis, 1984; Arioglu and Ersoy, 1987).

Mixtalol was applied to cotton, wheat, potatoes and corn, and yield increases were obtained. Experience from India and overseas trial indicates that two applications of Mixtalol are most effective in increasing yield of most annual crops. The effect of Mixtalol is to stimulate dry matter production (Anonymous, 1986).

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The objectives of this research to use Mixtalol at different nitrogen levels in soybean and to investigate of the effect on soybean.

Material and methods: This experiment was conducted as a second crop after wheat harvesting in the experimental field of the University of Cukurova in 1986. In this research "Amsoy 71" soybean variety and "Mixtalol" growth stimulator were used. Mixtalol is supplied as a 1% emulsion of active ingredients, equivalent to 10,000 ppm. This was to be diluted.

Maximum air temperature increased up to 39.5° C by the end of the growing period. The average air temperature was varied between 21.9° C and 27.9° C during the growing period. Also, 10cm soil temperature varied between 24.5° C and 33.4° C. Sufficient water was supplied by irrigation to maintain soil moisture higher than 50% of the water-holding capacity at all times.

The soil type of experimental area is sandy-loam and soil pH ranged between 7.5 and 7.8. Soil organic matter is low and it contains a high amount of lime (özbek et al., 1974).

The experimental design was a randomized complete block arranged in a split-plot with four replications. The main plots were 0, 30 and 60 kg ha^{-1} nitrogen for N_0, N_1 and N_2, respectively, and the subplots were 0, 1,2 and 5 ppm Mixtalol for M_0, M_1, M_2 and M_3 respectively. The seedbed was prepared with disc harrow after wheat harvesting. 60 kg ha^{-1} P_2O_5 was broadcast at the time of soil preparation to all plots. The plot sizes were 4.2 m X 5.0 m = 21.0 m^2 and row spacing was 70 cm. The seed was inoculated with *Rhizobium japonicum* and planted by hand on June 24, 1986. After planting, all plots were irrigated to obtain a uniform emergence of stand. Mixtalol was applied with 'knapsack sprayer' at the V_2 (plants are 10 to 15 cm tall and three nodes have leaves with completely unfolded leaflets) and R_2 (open flower at one of the two uppermost nodes on the main stem with a fully developed leaf) growth stages. The experimental area was furrowed twice during plant development to control weeds and the irrigation was applied by flood irrigation system four times. Plots were harvested by hand and threshed by a stationary plot thresher.

Plant height, pod number, the lowest pod height, thousand-seed weight and seed yield characteristics were investigated in this research.

Results and discussion: Results are presented in Table 1. The plant height increased with the Mixtalol application in each nitrogen level. It varied between 85.56 cm and 101.70 cm. The plant height was highest in N_2M_3 application.

Lowest pod height varied between 13.9 cm and 21.0 cm. The lowest pod height increased with the nitrogen level was increased.

Highest thousand-seed-weight data was obtained in N_0M_3 application.

Results of the experiment showed that the soybean yield generally decreased when nitrogen level was increased. The most significant effect of Mixtalol was obtained at zero nitrogen level.

The soybean seed yield was 3537 kg ha^{-1} in zero nitrogen level with no Mixtalol application. On the other hand, seed yield reached to 3834 kg ha^{-1} in zero nitrogen level with 1 ppm Mixtalol application. 2 and 5 ppm applications of Mixtalol in the same nitrogen level had no significant effect on seed yield. Mixtalol effect decreased with increasing amount of nitrogen. The effect of nitrogen and Mixtalol treatment on soybean yield are shown in Figure 1.

Table 1. Influence of nitrogen (N) and Mixtalol (M) on soybean seed yield, plant height, lowest pod height, pod number and 1000-seed weight

Nitrogen (N) and Mixtalol (M) treatment	Plant height (cm)	Lowest pod height (cm)	Pod number (num./plant)	1000-seed weight (g)	Seed yield (Kg/ha)
N_0M_0	85.76	13.90	36.40	175.30	3538.0
N_0M_1	85.56	14.52	34.23	176.30	3834.0
N_0M_2	86.60	16.20	31.80	176.50	3487.0
N_0M_3	91.34	18.05	36.22	180.10	3673.0
N_1M_0	92.86	15.99	32.96	171.40	3429.0
N_1M_1	94.13	16.70	34.05	168.90	3343.0
N_1M_2	98.45	18.70	35.84	174.50	3429.0
N_1M_3	100.37	18.19	33.04	173.40	3509.0
N_2M_0	97.61	18.91	29.95	163.20	3309.0
N_2M_1	97.52	19.29	33.71	171.50	3350.0
N_2M_2	99.70	19.56	32.17	174.30	3313.0
N_2M_3	101.70	21.00	32.96	169.90	3384.0
CV (N) %	5.85	6.58	5.83	3.92	7.71
CV (M) %	6.23	7.34	8.79	3.20	5.71
L.S.D. (5%)	8.87	1.90	4.09	8.67	338.40

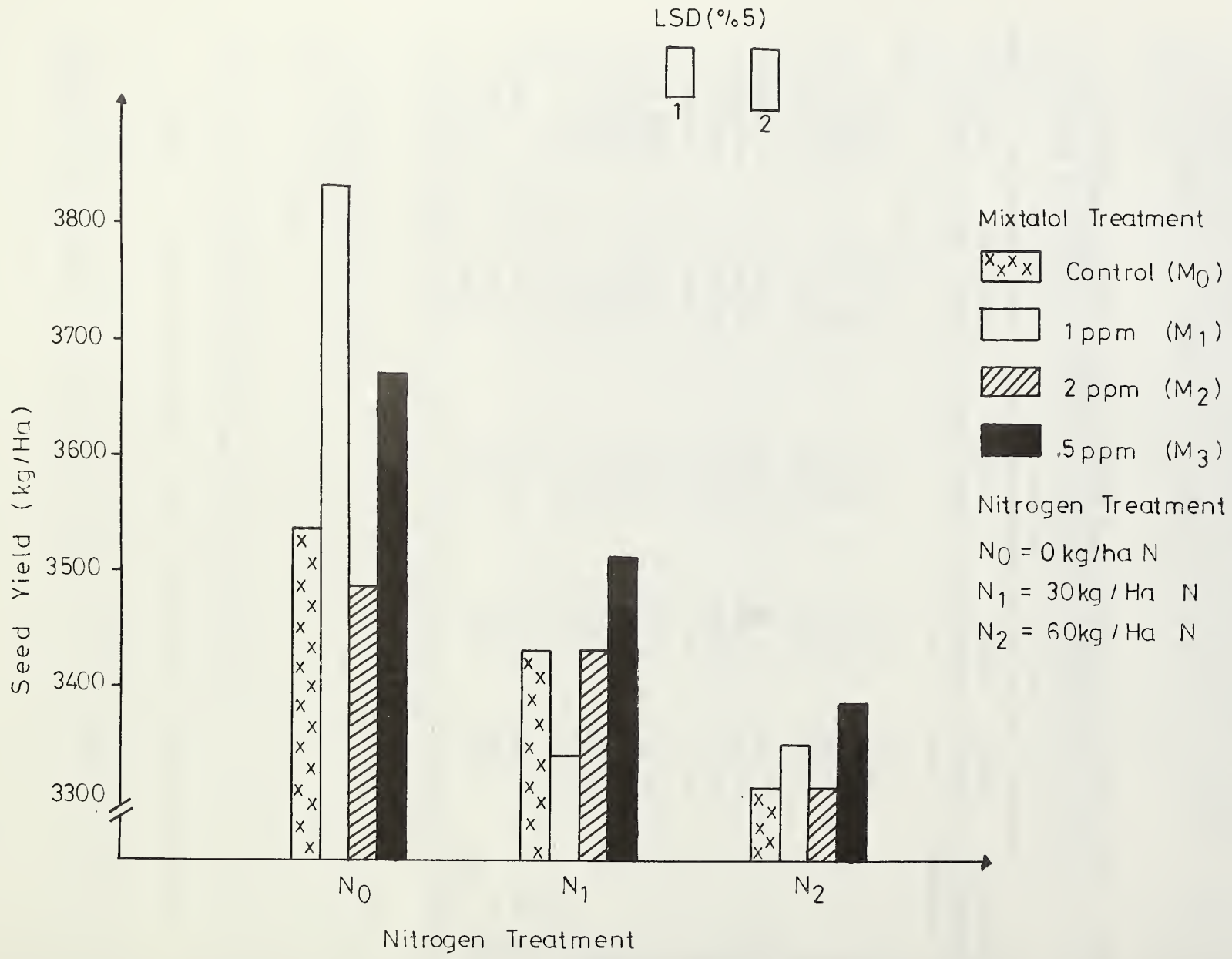

Fig. 1. Effect of Nitrogen and Mixtalol Treatment on Soybean seed Yield

1.) $N_1 M_1 - N_1 M_0$ 2) $N_1 M_1 - N_0 M_1$ or $N_1 M_1 - N_0 M_0$

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1) The effect of colchicine during mitosis in soybean.//

This study was conducted to investigate the changes that occur in cytoplasm and the formation and inhibition of microtubules at the ultrastructural level by analyzing all the stages of cell division in both control and colchicine-treated root tip meristematic cells of Glycine max L. Merr.

The inhibiting effect of colchicine upon microtubules has been known for a long time. The thought that investigation of these effects would be a great contribution to cancer research made many scientists concentrate upon this subject. In the 1960's the effect of colchicine on cell division was investigated, but remained at the level of light microscope. There was also some research upon this subject after the invention of the electron microscope.

Ledbetter and Porter (1963) were the first scientists who ultrastructurally investigated the structure of microtubules in telophase of meristematic cells of Triticum vulgare. Then, there were studies investigating the structure of microtubules by many scientists. Bajer (1973) tried to examine the interaction of microtubules and the mechanism of chromosome movement. Lambert (1980) investigated the relation of spindle with nuclear membrane and chromosomes during the formation of anaphase in Haemanthus albiflos, H. katharinae and Mnium hornum. Tippit et al. (1980) examined the organization of microtubules and classified them in Ochromonas danica. Hawes (1981) examined the mitosis and cytokinesis in maize roots by using low and high voltage microscopes. Fowke et al. (1974) investigated the ultrastructure of cultured cells and protoplasts of soybean during cell division but could not provide much clarification of the subject. Wilson (1970) examined the mitotic changes on the root cells of Allium cepa. Mesquita (1966) investigated the modifications in meristemic cells, mitochondria and endoplasmic reticulum of roots of Allium cepa caused by the effect of colchicine and David (1968) examined the changes on the mitochondria by the toxic compounds.

Material and methods: 'Chippewa' type soybean seeds used as research material. When germinated they were classified into two groups when their roots reached a length of 0.5mm. The first group was left to germinate at 25-27°C while the second was put in a 0.1% colchicine solution for 3 h at room temperature. Then the seeds were washed with distilled water and left to germinate for 24 h before starting the fixation process.

Root tips were fixed in 1% gluteraldehyde + 0.5% paraformaldehyde (Karnovsky, 1965) in 0.1M phosphate buffer at pH 6.8 for an hour; followed by post-fixation in 1% OsO_4 for 2 h; dehydrated in a graded alcohol series and embedded in Epon 812. Sections were cut at 60-90 nm on a Reichert ultramicrotome OmU_4 using glass knives, stained with Sato's (1967) lead citrate enhanced with uranyl acetate and viewed in a Hitachi HS-9 transmission electron microscope at 75kV.

Results and discussion: The nucleus was seen to occupy the large part of the cell during the metabolic stage. In this stage, circular microtubule sections were found in the regions near to cell walls. Galatis et al. (1984) made same observation upon the preprophase-band microtubules of two Triticum species and claimed that the preprophase-band microtubules play an important role in the formation of the mitotic stage microtubules. Fowke et al. (1974) said that there were no preprophase microtubules after his studies on soybean tissue cultures. But in our experiments, there were observed circular microtubule sections similar to those preprophase microtubules but they were in very low numbers at the start of the prophase. There were no structures observed in the following stages. It is highly possible that these play an important role in the formation of other microtubules.

During the early stage of prophase, the number of cytoplasmic organelles was increased compared with interphase. At mid prophase, the nuclear envelope was partly broken, especially at the pole regions. In late prophase, chromosomes had begun to take shape, the nuclear envelope was broken, the microtubules entered the karyoplasm with a few endoplasmic reticulum and cytoplasmic material after the puncture of the nuclear envelope.

During the early stage of metaphase, the chromosomes were completely shaped. The microtubules reached their maximum number before the rearrangement of chromosomes along the equatorial plate occurred. At this stage, the plastids and other cytoplasmic organelles gathered at the pole regions in the zones near the chromosomes. Different types of microtubules were found at that stage. Tippit et al. (1980) investigated the microtubules of Ochromonas danica and categorized and named the microtubules as follows: 1. Free microtubules, 2. Interdigitated microtubules, 3. Polar microtubules, 4. Kinetochoral (chromosomal) microtubules. We classified the locations and the arrangements of the mitotic apparatus microtubules after the analysis of all the stages of soybean mitosis as follows: 1. Kinetochoral (chromosomal) microtubules, 2. Free microtubules, 3. Continuous or inter-polar microtubules.

During anaphase, the mitochondria, plastids, endoplasmic reticulum, and other cytoplasmic organelles were concentrating at the pole region during the anaphase stage.

In the telophase stage was the high number of mitochondria around the cell plate. Apart from that, a few dictyosomes were also determined around the cell plate information. The phragmoplast microtubules overlapped each other and were buried in the electron-dense granular material between the phragmoplast vesicles. Hawes et al. (1981), after his studies on Zea mays mitosis, advocated that phragmoplast formation results from the fission of vesicles formed by dictyosomes following anaphase. In spite of these claims by these workers, dictyosomes were very rarely observed in the interzonal region during telophase.

During the colchicine treatment, it was observed that the chromosome action came to a close, the cell division remained constant at the metaphase stage and consequently resulted in polyploidy. The cytoplasm was damaged when compared with control cells. It was also determined that the most affected organelle in the cytoplasm was the endoplasmic reticulum lamella system. The endoplasmic reticulum consisted of an interesting looking enlarged lamella system not seen in control cells.

The lamellae also appeared to be enlarged and caused vacuolation. The lamellae systems mentioned above were dominated not by a typical granular-nature endoplasmic reticulum, but by a typical agranular-nature endoplasmic reticulum. The appearance of the mitochondria was also very interesting. These organelles were swelled. There were also mitochondria that had distinguishable double membranes but totally destroyed inner membrane systems. The root cells treated with colchicine did not contain dictyosomes as seen in control cells. Various mutations seen in the membrane system of cytoplasm also appeared in the dictyosomes. Also, there were various sized irregular vacuoles in the cytoplasm. David (1969) made the same observations. Lambert (1980) observed the inhibition of microtubule formation in cells treated with colchicine for a long time and called this situation "C-metaphase" in his study on the endosperm of Haemanthus treated with 0.4% colchicine. Apart from that, he also observed the separation of sister chromatids called "C-anaphase" in the cells which he treated with 0.5% colchicine. In our results, we observed the phenomona of "C-metaphase", but there was a polyploid situation in the chromosomes. The stage of "C-anaphase" was not observed in our study.

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1) Cytological evidence for maternal inheritance of plastid DNA in the genus Glycine subgenera Glycine and Soja.

In the past eight decades, genetic evidence has been presented for the pattern of plastid inheritance in some 60 genera of angiosperm genera (reviewed by Kirk and Tilney-Bassett, 1978; Sears, 1980). Two-thirds of these genera appear to have maternal inheritance of plastids.

There is solid genetic evidence for the maternal inheritance of plastids in the cultivated soybean, Glycine max (L.) Merr. (Terao, 1918; Palmer and Mascia, 1980; Shoemaker et al., 1985), and restriction fragment length polymorphisms (RFLPs) have been used to infer the maternal inheritance of plastid DNA in the annual wild soybean, G. soja Sieb. & Zucc. (Hatfield et al., 1985). However, there have been no reports on the plastid inheritance patterns among the wild perennial soybean species that belong to the subgenus Glycine.

Recently, we reported on a DNA-fluorochrome/epifluorescence microscopy protocol that permits rapid screening for plant species potentially capable of biparental transmission of plastid DNA (Coleman et al., 1986). When pollen from plant species known genetically to have the biparental mode of plastid transmission was examined with this cytological method, plastid DNA aggregates were observed in the cytoplasm of the male reproductive cells, i.e., the generative and/or sperm cells. For species known to display maternal inheritance, no such DNA aggregates were detected. Initial cytological observations on pollen from G. max and G. soja, which were reported last year (Corriveau, 1987), corroborated the known genetic evidence. No plastid DNA was observed in the generative cells of pollen collected from these two species.

The purpose of the study presented here was to use the DNA-fluorochrome/epifluorescence protocol on pollen samples from cultivars and accessions of the genus Glycine Willd., subgenera Glycine and Soja (Moench) F. J. Herm., to determine if lines, or species, are potentially capable of paternal transmission of plastid DNA. The subgenus Soja includes the cultivated soybean (G. max) and its wild counterpart (G. soja). From the subgenus Glycine, which contains the wild perennial soybean species, we examined pollen from G. canescens F. J. Herm., G. clandestina Wendl., G. crytoloba Tind., G. falcata Benth., G. latifolia (Benth.) Newell & Hymowitz, G. microphylla (Benth.) Tind., G. tabacina(Labill.)Benth., and G tomentella Hayata.

Materials and methods: Soybean plants were grown in pots in the greenhouse facilities at Brown University. Mature pollen grains were collected from flowers and either used immediately, or fixed in 95% ethanol:glacial acetic acid (3:1), and subjected to cytological analysis as described previously by Coleman and Goff (1985). Living pollen grains were stained with 0.125 µg/ml 4',6-diamidino-2-phenylindole (DAPI) in a "chopping buffer" (15 mM Tris pH 7.4, 2mM NaEDTA, and 80mM KCl), and fixed pollen was stained with 0.05 µg/ml DAPI in McIlvaine's buffer (pH 4). DAPI-DNA fluorescence was revealed by using a Zeiss AXIOPHOT epifluorescence microscope equipped with a 50 W mercury lamp to deliver excitation light, and the Zeiss 48-77-02 combination

of excitation and emission filters. DNase-treated controls served to monitor the specificity of staining for DNA. Pollen from plant species were scored as being potentially capable of transmitting plastid DNA if DNA aggregates were observed in the generative or sperm cells of mature pollen. If no such DNA aggregates were detected, the pollen was scored as representing maternal transmission of pastid DNA. At least 100 pollen grains from at least two plants were scored for each soybean cultivar or accession.

Results: The cytological evidence obtained from G. max, G. soja, and the eight species of wild perennial soybean (Table 1) indicates that plastid DNA is maternally inherited not only in the subgenus Soja as previously reported (Hatfield et al., 1985; Shoemaker et al., 1985), but also in the subgenus Glycine. In none of the pollen grain preparations examined were DNA aggregates detected in the cytoplasm of the generative or sperm cells.

Worthy of mention is the observation that, whereas the mature pollen grains from G. max and G. soja are binucleate, those from the eight species of wild perennial soybean are trinucleate (Table 1).

Discussion: Our lab has been developing the use of DNA-fluorochromes in conjunction with epifluorescence microscopy for in situ detection of plastid DNA in pollen. We have detected plastid DNA aggregates in the generative and/or sperm cells of nine plant species known genetically to display biparental inheritance of plastids, and not in 25 species known to follow the maternal mode (Corriveau and Coleman, 1988) The striking correlation between the cytological results and known genetics is indicative of the value of utilizing this rapid screening method to obtain cytological evidence for the potential mode of plastid DNA inheritance from plant species for which inheritance patterns have been reported.

The DNA-fluorochrome/epifluorescence microscopy protocol was used on pollen from a range of soybean species for several reasons. First, no members of the subgenus Glycine have ever been examined for the mode of plastid transmission. Second, we have obtained provocative cytological results that suggest that there is tremendous variability within the Fabaceae for the mode of plastid DNA inheritance (Corriveau and Coleman, 1988). Six out of 22 genera studies in the legume family are potentially capable of biparental inheritance of plastids. Furthermore, cytological evidence obtained from accessions of Pisum indicated that there might be variation within and between species of this legume genus for the pattern of plastid DNA inheritance. And finally, there is genetic evidence for variability within species with regard to plastid inheritance patterns in Oenothera and Pelargonium (Tilney-Bassett and Abdel-Wahab, 1979).

Soybean breeders seeking to manipulate plastid genomes now have the expectation that these eight species of wild perennial soybean, as well as the annual cultivated and wild counterpart species, will display maternal inheritance of plastid DNA. The findings reported here are especially significant in light of the reports on interspecific hybrids within the subgenus Glycine (see Singh and Hymowitz, 1985), and even interspecific hybrids between the subgenera Glycine and Soja (see Newell et al., 1987).

Finally, the observation that mature pollen from species of the subgenus Soja are binucleate while those of the subgenus Glycine are trinucleate is of interest for several reasons. This is the first report of trinucleate pollen

from a member of the tribe Phaseoleae, and Glycine is only the eleventh genus reported to contain species with both types of mature pollen grains (Brewbaker, 1967). The overwhelming majority of angiosperm genera are monotypic with regard to pollen cytology. Recent advances with interspecific hybridizations between the subgenera Glycine (trinucleate pollen) and Soja (binucleate pollen) would seem to warrent an investigation to determine whether or not there is a simple Mendelian inheritance pattern to explain this trait.

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Table 1. List of cultivars or accessions from the genus Glycine subgenera Glycine and Soja assessed for potential paternal transmission of plastid DNA.

Taxon	Cultivar or accession	Pollen cytology*	Cytological evidence for mode of plastid inheritance
Subgenus Glycine			
Glycine canescens	399478	III	maternal
G. clandestina	246590	III	maternal
G. cyrtoloba	373993	III	maternal
G. falcata	246591	III	maternal
G. latifolia	321394	III	maternal
G. microphylla	CSIRO 1143	III	maternal
G. tabacina	272099	III	maternal
G. tomentella	373980	III	maternal
Subgenus Soja			
G. max	Arlington	II	maternal
	Clark	II	maternal
	Essex	II	maternal
	Hill	II	maternal
	Jilin No. 13	II	maternal
	Kent	II	maternal
	Peking	II	maternal
	Williams	II	maternal
	19986	II	maternal
	157469	II	maternal
	546082	II	maternal
	839454	II	maternal
G. soja	407203	II	maternal

*III = mature pollen is trinucleate, II = mature pollen is binucleate

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1) Plant regeneration from soybean callus cultured on media containing NaCl.

Legumes have long been recognized to be either sensitive or moderately resistant to salinity (Maas and Hoffman, 1977). Within soybeans, studies indicate that there are differences in salt tolerance among cultivars. Lauchli and Wieneke (1979) showed that the soybean variety 'Lee' is more salt tolerant than the variety 'Jackson'. Tissue culture may be useful in plant improvement for the production of salt-tolerant plants through selection of salt-tolerant cell lines and the regeneration of plants from these lines (Smith and McComb, 1981; Nabors et al., 1975, 1980). For most pulse crops, plant regeneration has been difficult; however, induction of somatic embryogenesis in soybean has been reported by Ranch et al., 1985, Ghazi et al., 1986 and others.

In this paper we report on the effect of NaCl on in vitro callus development and regeneration of soybean plantlets.

Materials and methods: Immature embryos were obtained from pods of 'Prize' soybean plants grown in a greenhouse. One- to two-cm long segments of pods containing a single immature embryo were surface sterilized in 75% ethanol followed by stirring under vacuum for 10 minutes in 5% commercial bleach plus Tween 20. The pod segments were then rinsed three to four times with sterile deionized water. The immature embryos, 4 to 10 mm long, were removed from the pod and the cotyledons excised (Ghazi et al., 1986). The cotyledons were placed on a semi-solid basal medium containing LS (Linsmaier and Skoog, 1965) major salts, minor salts and vitamins, 2% sucrose, 0.7% agar and 10 mg/l 2,4-D, with NaCl at 1, 3, 5, 7 or 9 g/l.

Embryo-like structures developed on callus derived from the cotyledonary segments within three weeks. These structures were counted, and transferred to a regeneration medium consisting of LS with 0.103 mg/l GA_3 + 0.132 mg/l ABA + 0.102 mg/l IBA and the same respective amounts of NaCl. After a two- to three-week incubation in the dark, shoots were produced. These shoots were subcultured in the light on half-strength LS medium containing the same NaCl levels as in the previous culture medium and containing 1.22 mg/l BA + 1.00 mg/l GA_3 for two weeks. The shoots were then transferred to the same medium without GA_3 for rooting. Sodium chloride concentrations were kept constant from culture initiation to plant regeneration.

Rooted plantlets were transferred to pots containing vermiculite, soil and sand and placed in a growth chamber. After establishment, the plants were transferred to larger pots containing soil and placed in the greenhouse.

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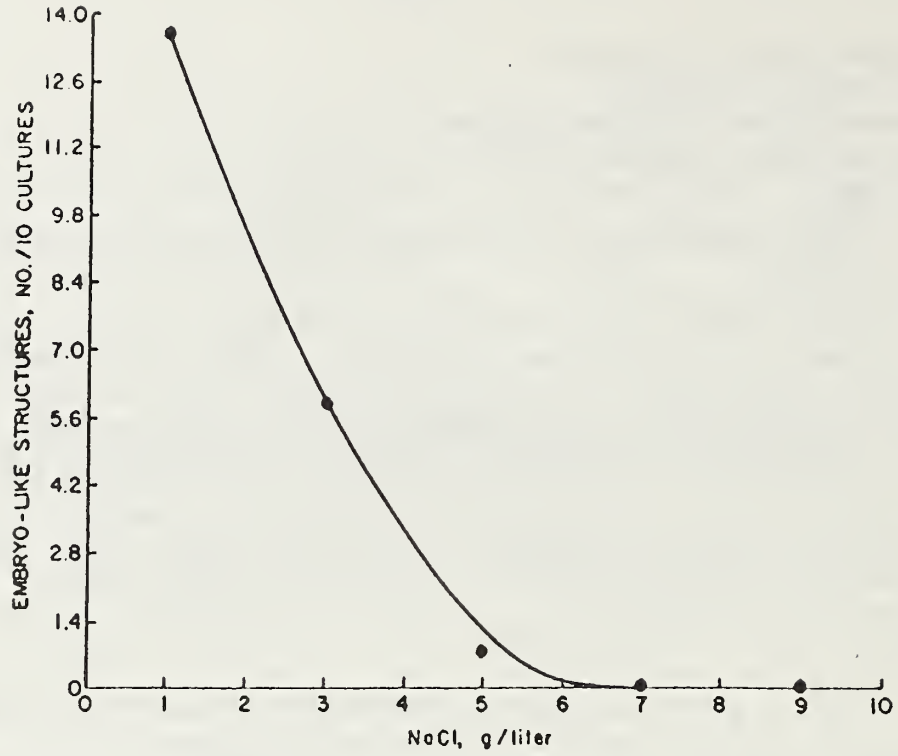

Figure 1. Influence of NaCl concentration on production of embryo-like structures, based on 2100 cultures. The F value obtained for regression, 191, was highly significant with 2 and 4 degrees of freedom.

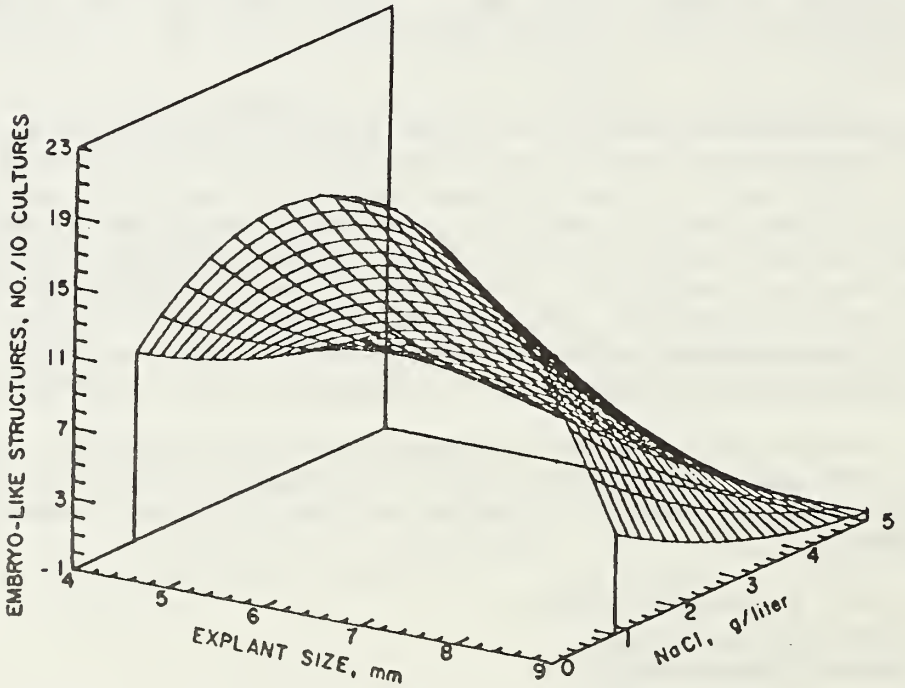

Figure 2. Influence of NaCl concentration and explant size on production of embryo-like structures, based on 1620 cultures.

Results and discussion: The procedure for plant regeneration in 'Prize' soybean has previously been reported (Ghazi et al., 1986). When cotyledons were cultured on media containing NaCl, the number of somatic embryos produced was inversely proportional to the concentration of NaCl (Fig. 1). From cultures grown on media containing 1 g/l NaCl, 25 plants were regenerated. These plants matured and set seed. Additionally, five plants were recovered from cultures grown on 3g/l NaCl, but they did not mature. A few embryos were produced on 5 g/l NaCl, but none developed into plants. At 7 and 9 g/l NaCl, the cultures failed to produce embryo-like structures.

A second factor observed in this experiment was the effect of cotyledon size. On 1 g/l NaCl, the optimum size for formation of somatic embryos was 6 mm (Fig. 2). As salt concentration increased, the effect of cotyledon size was eliminated.

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1) Soybean oil stability: Effect of genotype, environment and heat denaturation of seed components.

Oxidative stability of crude soybean oil samples was assessed by determining the induction period for peroxide value formation of oil samples incubated at 60° C. Large genotypic and environmental effects were seen for oil stability.

Materials and methods: Forty-three soybean cultivars of diverse parentage and two plant introductions (PI) lacking lipoxygenase-1 (Hildebrand and Hymowitz, 1981) were analyzed for oil stability (Table 1). All accessions were grown in Urbana, IL, in 1980 except cultivars 'Bedford', 'Biloxi', 'Forrest', 'Hardee', PI 133226 and PI 408251, which were grown in Stoneville, MS in 1980. In addition, seed from the cultivar 'Williams' grown at four locations in Illinois in 1980 and grown at two locations in 1979 (Table 2) were analyzed for oil stability. Oil from commercial sunflower hybrids grown at Brownstown, IL, in 1980 were included for comparison studies.

Duplicate 15-g seed samples (adjusted to 12-13% moisture) from each seed lot were ground 1 min in a Mitey-Mill grinder (Sturdee Health Products, Island Park, NY). Dehulling soybean seeds prior to the extraction of oil did not affect stability. Thus, the studies reported herein are for undehulled soybeans. Only hand dehulled sunflower seeds were used in oil stability studies (included for comparison to the soybean oil samples). The resulting meal was placed in glass bottles and 15 ml hexane were added 30 min after grinding. The bottles were covered with aluminum foil, capped, and stored at 4° C for 48 hr with frequent periodic vigorous shaking, after which the hexane supernatant was allowed to separate. The hexane extracts then were filtered through Whatman No. 50 filter paper and centrifuged at 20,000 x g for 20 min. The hexane-extract supernatants from centrifugation were transferred to 25-ml vials and the hexane evaporated with stream of nitrogen within a glove bag (I^2R, Cheltenham PA). The vials then were sealed with rubber septa in the nitrogen atmosphere and stored at -17°C.

Measurement of the induction period for peroxide value (PV) formation at 60°C was used to assess oil oxidation (Odumosu et al., 1979; Kaitaranta and Ke, 1981; St. Angelo et al., 1975).

The phospholipid content of the oil samples was determined by a modified method of Eng and Noble (1968). The phosphate (Pi) content of oil digests was determined by a modified Murphy-Riley procedure (John, 1970).

The fatty acid (FA) composition of oil samples, stored at -17°C under nitrogen, was determined using GLC analysis of methyl esters (Chaven et al., 1982). Free fatty acids (FFA) of the oil samples were determined using GLC analysis of the FFA after they were separated from the major oil components on Whatman linear-K preadsorbent silica-gel TLC plates, (plate type K5W, Pierce Chem. Co.) with a hexane-diethylether-acetic acid (80:20:1;V,V,V,) solvent system containing 0.01% BHT. Tocopherol composition was determined of oil samples extracted as above using high performance liquid chromatography (HPLC). An Ultrasphere silica column (4.6 x 250 mm) was used with a mobile phase of 1.2% isopropyl alcohol in hexane and the flow rate was 1.33 ml min^{-1}.

Table 1. Induction period of peroxide value formation of accessions used in the oil stability studies.

Accession	Induction period[a]	Accession	Induction period
Aksarben	6.5 ± 1.1	Hoosier	6.5 ± 1.1
Altona (sp_1)	8.7 ± 1.5	Hong Kong	7.0 ± 1.4
Amsoy 71	7.3 ± 1.2	Kabott	6.5 ± 1.2
Bansei	6.5 ± 1.2	Kanum	6.8 ± 1.4
Bedford	8.5 ± 0.8	Kingston	6.4 ± 1.5
Biloxi	7.8 ± 0.5	Manchu (Lafayette)	10.4 ± 1.6
Black Eyebrow	9.5 ± 1.4	Manchuria	6.5 ± 1.1
Boone	6.5 ± 1.2	Morse	6.7 ± 1.3
Chestnut	9.2 ± 1.0	Norredo	6.5 ± 1.2
Earlyana	10.6 ± 2.2	Ontario	8.7 ± 0.2
Ebony	8.6 ± 1.5	Osaya	8.8 ± 1.2
Elton	6.5 ± 1.3	Sanga	6.5 ± 1.3
Emerald	6.6 ± 1.3	Shington	10.4 ± 1.6
Etum	6.5 ± 1.2	Sooty	5.3 ± 0.7
Forrest	7.8 ± 1.1	Sousei	6.7 ± 0.7
Fuji	6.1 ± 1.8	Toku	6.5 ± 1.4

[a]Days to reach a peroxide value of 100.

Table 1 (cont'd)

Accession	Induction period[a]	Accession	Induction period
Goku	6.5 ± 1.3	Verde	7.3 ± 1.1
Green and Black	10.4 ± 1.7	Waseda	9.3 ± 1.9
Harbaro	6.6 ± 1.1	Wea	10.4 ± 1.6
Harbinsoy	6.4 ± 1.2	Williams	7.4 ± 1.1
Hardee	6.6 ± 0.1	Wilson 6	6.5 ± 1.2
Hidatsa	6.5 ± 0.1	PI 133226	6.7 ± 1.3
		PI 408251	7.0 ± 0.4

[a]Days to reach a peroxide value of 100.

Table 2. Effect of environment on phospholipid content and stability of oil extracted from Williams soybean seed.

Environment[a]	Induction period[b]	Phospholipid content (%)
Belleville 1980	6.7 ± 1.3	0.26 ± 0.01
Belleville 1979	4.7 ± 1.3	0.29 ± 0.02
Girard 1980	9.1 ± 2.2	0.38 ± 0.04
Eldorado 1980	8.0 ± 0.5	0.36 ± 0.01
Urbana 1980	7.4 ± 1.1	0.32 ± 0.01
Urbana 1979	8.1 ± 1.2	0.26 ± 0.01

[a]Locations in Illinois and year that the soybeans were grown.

[b]As in Table 1.

Table 3. Oil stability, % phospholipid (PL), % free fatty acids (FFA), and tocopherols contents of oil from autoclaved and unheated seeds.

Accession	Induction period[a]	PL %	FFA[b] %	Tocopherol, µg/g oil α	β	λ	δ	Total
Altona (*sp*$_1$)	8.7±1.5							
Altona (*sp*$_1$) autoclaved[c]	20.5±2.3							
Amsoy 71	7.3±0.5	0.85±0.16	0.16±0.02	177±29	12±1	582±57	55±7	826±94
Amsoy 71 autoclaved	21.0±0.6	2.23±0.06	0.16±0.04	134±15	16±1	629±23	52±1	831±40
Chestnut	9.2±1.0	0.15±0.03	0.15±0.03	119± 1	9±1	516±10	38±3	681±11
Chestnut autoclaved	19.5±1.0	2.19±0.04	0.21±0.04	119± 3	13±1	459±16	32±2	623±19

[a]As in Table 1.

[b]Free fatty acids as % weight of oil.

[c]Seeds autoclaved 1 hr prior to extraction of oil.

Table 3 (cont'd).

Accession	Induction period[a]	PL %	FFA[b] %	Tocopherol, µg/g oil α	β	λ	δ	Total
Williams	7.4±1.1	0.27±0.02	0.24±0.01	92± 4	7±3	583±10	61±1	743± 4
Williams autoclaved	20.5±2.7	2.50±0.50	0.25±0.02	112±12	15±2	602±26	55±1	784±41
Commercial soy oil[d]	11.3±2.1							
Sunflower[e]	6.0±1.1	0.04±0.01						
Sunflower autoclaved	8.5±1.6	0.43±0.01						

[d]Commercial partially hydrogenated soybean oil obtained at a local grocery.

[e]Oil from mixed seeds of commercial sunflower hybrids (oil type) grown at Brownstown, IL in 1980.

Results: The 43 accessions evaluated showed induction periods ranging from 5.3 to 10.6 with a mean of 7.5 days (Table 1). The accessions or their parents were introduced into the U. S. from China, Japan, Korea, or the U. S. S. R. There was no significant relationship between country of origin and induction period. Stability of oil from PI 133226 (Indonesia) and PI 408251 (Korea) was similar to that of the other four accessions (Bedford, Biloxi, Forrest, Hardee) grown at Stoneville, MS. Cultivar Williams grown in six environments showed induction periods ranging from 4.7 to 9.1 with a mean of 7.3 days (Table 2). Commercial refined soybean oil (including partial hydrogenation) showed greater oxidative stability than a typical unrefined soybean oil (Table 3). Autoclaving soybean seeds prior to extraction of the oil resulted in an oil having about three times greater stability (Table 3). Oil from autoclaved soybean seeds also has much greater stability than commercial refined soybean oil.

No significant correlation was found between induction period and linolenic acid content, FFA, initial PV or phospholipid content among the accessions analyzed. The correlation of induction period and phospholipid content of Williams seed from six environments was 0.53 (Table 2). Oil from autoclaved soybeans had similar FFA levels as did oil from unheated soybean seeds (Table 3). Phospholipid content of oil from autoclaved soybeans showed a dramatic increase, greater than 10-fold in some cases (Table 3). Phoshpolipid content of oil from autoclaved soybeans showed a dramatic increase, greater than 10-fold in some cases (Table 3). Fatty acid composition of total FA and FFA was not affected by autoclaving the seeds (Table 4). The FFA had a higher content of saturated fatty acids than the total oil (Table 4).

Soybean oil (Table 3) and soybeans seeds (Table 5) were found to contain detectable quantities of alpha-, beta-, gamma-, and delta-tocopherol with gamma-tocopherol predominant. Low levels of gamma-tocotrienol sometimes were detected. The values shown (Tables 3 and 5) for beta-tocopherol may be somewhat higher than the actual levels due to unidentified conditions coming off the column at nearly the same time and the low actual levels of beta-tocopherol. The soybean oil showed genotypic differences in both total tocopherol content and the distribution of the individual tocopherol isomers (Table 3). These differences were less pronounced with the tocopherols of the whole seed (Table 5). All tocopherol isomers showed similar levels in oil from autoclaved and unheated soybean seeds (Table 3). However, all four of the tocopherol isomers showed higher levels in meal from autoclaved whole seeds than from unheated whole seeds (Table 5).

Discussion: Large differences in oil stability were seen among the 43 accessions grown in one environment (Table 1). Seed of a single accession grown in six environments showed differences in oil stability nearly as large (Table 2). Sample-to-sample variation also was quite high in some cases, as evidenced by the large standard errors. The accessions evaluated vary widely in maturity. Thus, there were some environmental differences among the accessions during seed filling and maturation that may have affected oil stability. The only major effect on oxidative stability of the oil was the autoclaving treatment, which would have inactivated heat-labile factors such as enzymes as well as changing some physical characteristics of the seed.

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2) Preliminary study on the inheritence of brown stem rot resistance in PI 86150 and PI 423930A.

Resistance to the fungal pathogen, Phialophora gregata (Allington and Chamberlain) W. Gams, which causes brown stem rot (BSR) of soybean, has become an increasingly desirable characteristic in Midwest USA breeding programs. The fungus invades through the roots, causes internal stem browning and interveinal foliar chlorosis and necrosis in more severe cases. Yield loss estimates range from 12 to 44%. All currently released public soybean cultivars with BSR resistance have a common ancestor PI 84946-2 which has the Rbs1 gene and possibly a minor gene for resistance (Sebastian and Nickell, 1985). PI 437833 has been shown to have a single dominant gene, Rbs2, for BSR resistance which exhibits duplicate dominant epistasis with Rbs1 (Hanson et al., 1988).

Genetic information on BSR resistance found in other strains (Nelson et al., 1988) would be an asset to designing breeding program strategies. The objective of this study was to examine the inheritance of BSR resistance in PI 86150 and PI 423930A.

Materials and methods: Screening of the material was conducted on the University of Illinois Agronomy and Plant Pathology South Farm at Urbana in 1986 and in the greenhouse in the spring of 1987. The field screening inoculation method was an adaptation of the greenhouse root-dip method reported by Sebastian et al. (1985) with the following modifications. The P. gregata isolate Cr2 was used, obtained from Dr. L. E. Gray, Univ. of Illinois, USDA-ARS. Inoculum concentration was increased to 1.5×10^6 propagules ml^{-1} (conidia and mycelial fragments) and volume was increased to 15 ml per plant. Methyl cellulose concentration was decreased to 0.75% (w/w). Twelve-day-old seedlings from F2 and parental seed were transplanted to the field in hills of three plants on 76 x 76 cm centers. Holes for transplanting were made with a 7 cm diameter bulb planter 10 cm deep. A trickle irrigation system was used to irrigate the hills immediately after transplanting and as needed throughout the duration of the experiment to maintain moist soil within 3 cm of the surface.

Plants from a random sampling of hills were rated when each reached growth stage R6 by counting the uppermost node displaying leaf symptoms. The stems of these plants were split completely to delineate resistant and susceptible BSR reactions. Those plants with scores within the 99% confidence interval of the stem or leaf symptoms mean of 'Cumberland' were considered susceptible. The stems of the remaining plants were carefully split in situ at the fifth node to determine the extent of stem browning. Virtually all of the plants could be classified and allowed to set seed.

Greenhouse screening was performed according to the procedure of Sebastian et al. (1985) with the following modifications. Inoculum concentration was increased to 1.2×10^6 propagules ml^{-1} and methyl cellulose concentration was decreased to 0.5% (w/w). Plants were rated at five weeks post-transplant for leaf and stem symptom severity separately using the following formula:

$$\text{Symptom severity} = \frac{\text{Top symptomatic node}}{\text{Total node number}} \times \text{\% of tissue in affected area necrotic}$$

Chi-square values were calculated for all populations and where the expected number of susceptible plants was five or less the modification of Cochran (1954) was used.

Results and discussion: Both the field and greenhouse screening methods were effective for determining BSR reactions. The greenhouse screening method was less variable and provided distinct leaf symptoms without appreciable confounding of leaf pathogen symptoms. Cumberland was highly susceptible and PI 84946-2, 'BSR 302', PI 86150 and PI 423930A were resistant to BSR in both the field and greenhouse (Table 1). Emphasis is placed on greenhouse leaf symptom results since the correlation between yield loss and leaf symptom severity is stronger than with stem symptom severity.

The stem and leaf reactions in the greenhouse of the F2 progeny of the cross PI 86150 X Cumberland fit a single dominant gene inheritance model for BSR resistance in PI 86150 (Table 1). The leaf and stem reactions in the greenhouse of the cross PI 86150 X PI 84946-2 fit a 63:1 for a single dominant gene in PI 86150 (Table 1) different from the Rbs1 and minor gene in PI 84946-2 (Sebastian and Nickell, 1985).

The stem and leaf reactions in the greenhouse of the cross PI 423930A X Cumberland fit a single dominant gene model for BSR resistance in PI 423930A (Table 1). The stem and leaf reactions in the greenhouse of the cross BSR 302 X PI 423930A fit a 63:1 ratio for dominant epistasis between the resistance genes of the parents (Table 1).

These results are inconclusive for three reasons. First, screening of F2:3 families has not been performed to confirm F2 results. Secondly, the inheritance of BSR resistance in BSR 302 has not been determined. Lastly, a small proportion of BSR 302 plants were susceptible in the field and greenhouse which could account for the apparent segregation. In that event, PI 423930A would possess the same gene for BSR resistance as BSR 302.

In contrast, PI 86150 and PI 84946-2 parental plants were all very BSR resistant in the greenhouse. This observation strengthens the inference that the susceptible reactions of F2 plants in the PI 86150 x PI 84946-2 cross were due to genetic segregation and not environmental variability.

PI 86150 and PI 423930A are both from Japan and so may have the same major gene for resistance. PI 423930A is glabrous and may be an undesirable source of BSR resistance, but genes for glabrousness and BSR resistance appear to segregate independently.

Table 1. Response of F_2 and parental soybean lines to *P. gregata*.

Cross or parent	Test loc.+	Basis of classification: Stem symptoms R	S	Ratio	X^2	P	Leaf symptoms R	S	Ratio	X^2	P
PI 86150 x Cumberland	G.H.	41	19	3:1	1.09	>.30	46	14	3:1	0.09	>.75
PI 86150 x PI 84946-2	G.H.	98	2	15:1	3.08	>.05	99	1	15:1	4.70	>.02
				63:1	0.003	>.95			63:1	0.003	>.95
PI 423930A x Cumberland	G.H	45	15	3:1	0.00	>.90	41	19	3:1	1.09	>.20
PI 423930A x BSR 302	G.H.	97	3	15:1	1.80	>.15	98	2	15:1	3.08	>.05
				63:1	0.57	>.20			63:1	0.003	>.95
Cumberland	Fld.	3	48				5	16			
	G.H.	0	20				0	20			
PI 86150	Fld.	48	0				18	0			
	G.H.	20	0				20	0			
PI 84946-2	Fld.	50	1				21	0			
	G.H.	20	0				20	0			
PI 423930A	Fld.	37	1				11	1			
	G.H.	20	0				20	0			
BSR 302	Fld.	51	3				24	0			
	G.H.	18	2				19	1			

+Fld. = field; G.H. = greenhouse.

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3) Effect of amino acid analogs and herbicides on the growth of organogenic soybean tissue cultures.

Selection in vitro can be a useful technique for possible crop improvement (Duncan and Widholm, 1986). Traits that can be selected for include herbicide resistance and amino acid accumulation. For crop improvement to be accomplished, however, plants must be regenerated from the selected lines and only recently has plant regeneration been accomplished with soybean tissue cultures (for example, Ranch et al., 1985). This report describes the growth inhibition of organogenic soybean cultures (Barwale et al., 1986) by three amino acid analogs and three herbicides and the effect of azetidine-2-carboxylate (A2C), a proline analog, on the formation of organogenic cultures from immature soybean embryos.

Materials and methods: Immature embryos from Williams 82 and A3127 (Asgrow) plants were cultured as described by Barwale et al. (1986) to produce embryogenic cultures. After several transfers, growth was determined by plating in duplicate or triplicate, five calli pieces

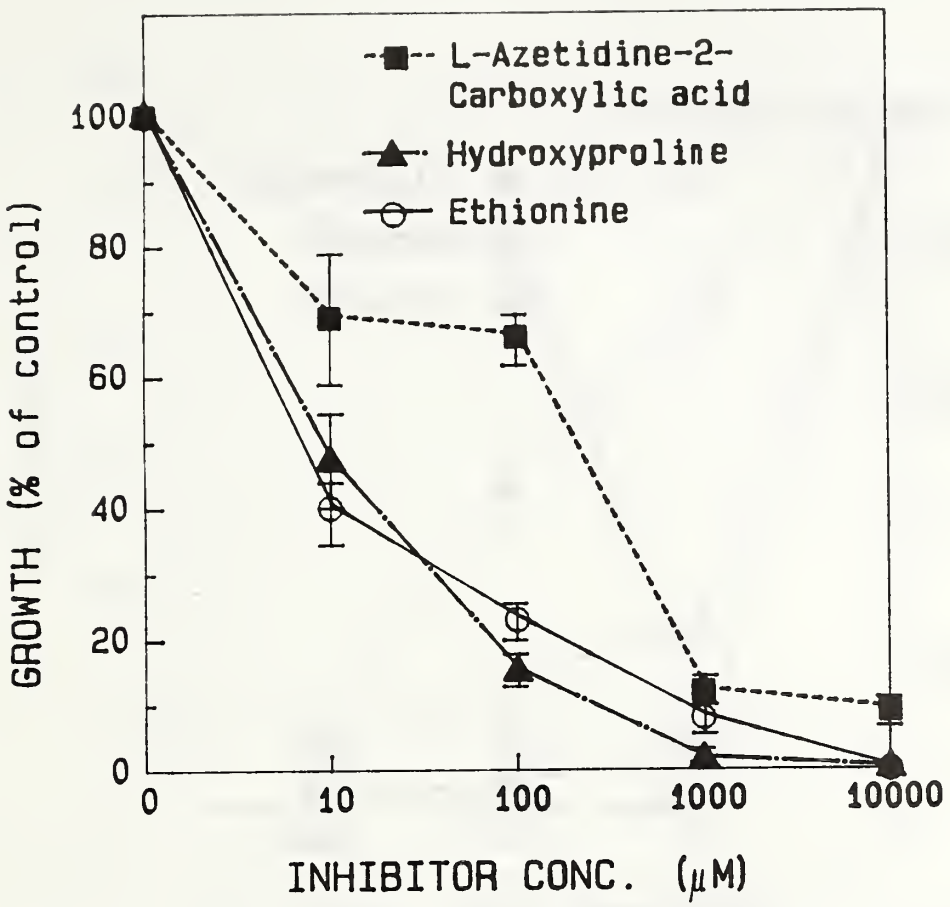

Figure 1. Effect of the amino acid analogs A2C, HPR and ETH on the growth of organogenic A3127 soybean callus during a 21-day incubation.

weighing a total of from 0.15 to 0.33 g fresh weight in 15 x 100 mm Petri dishes containing 30 ml of agar-solidified medium containing the inhibitors. The calli were weighed after incubating for 21 d at 27° to 28°C.

Immature embryos (two or three replicates of 15 embryos each) were plated on organogenesis medium containing A2C and the number that formed organogenic cultures was determined.

Results and discussion: Incubation of organogenic soybean callus with the amino acid analogs, A2C and hydroxyproline (HPR), both proline analogs and ethionine (ETH), a methionine analog, shows that complete inhibition occurs with about 1 mM and 50% inhibition near 10 to 100 μM (Fig. 1).

The herbicides glyphosate, atrazine and paraquat also are very inhibitory with paraquat giving nearly complete inhibition with 10 uM and

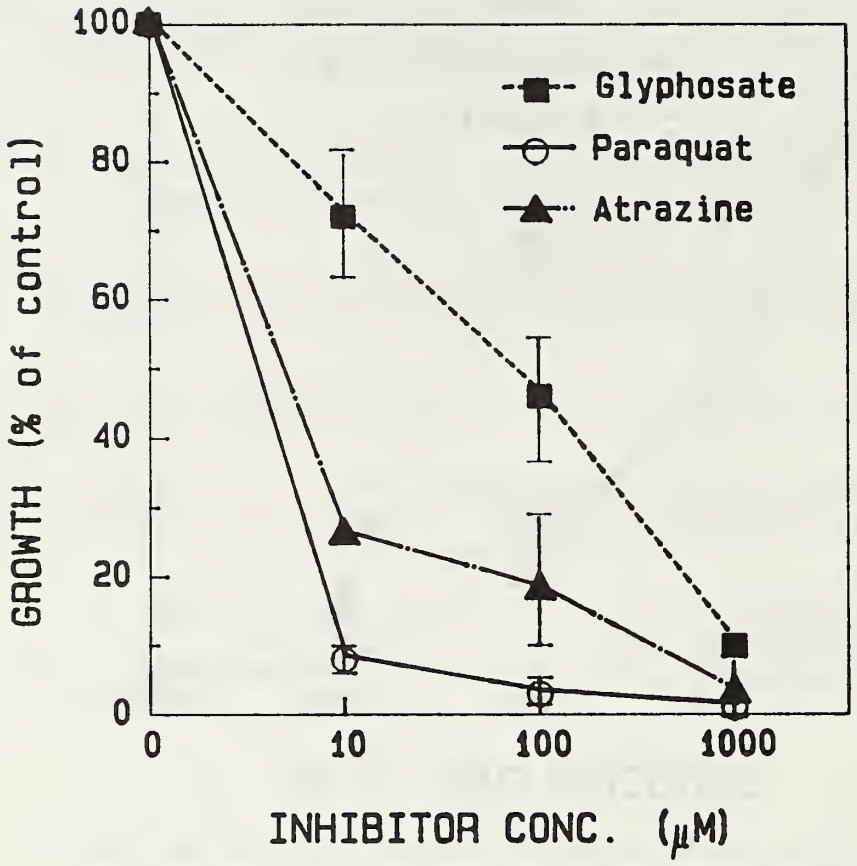

Figure 2. Effect of the herbicides paraquat, glyphosate and atrazine on the growth of orgarogenic A3127 soybean callus during a 21-day incubation.

glyphosate complete inhibition at 1 mM (Fig. 2). Atrazine inhibition was intermediate.

A2C also was very inhibitory to the formation of organogenic cultures when immature embryos were plated on initiation medium (Fig. 3). Complete inhibition occurred with 1 mM A2C while 100 μM showed only a slight decrease from the control. In these experiments with both Williams 82 and A3127 embryos, between 85 and 90% of the embryos formed organogenic cultures on the control medium without A2C.

These studies show that the three amino acid analogs A2C, HPR and ETH, and the herbicides paraquat, glyphosate and atrazine potently inhibit the growth of organogenic soybean cultures. A2C also inhibited the formation of organogenic cultures from plated embryos of two different genotypes. Thus one should be able to attempt selection experiments to produce

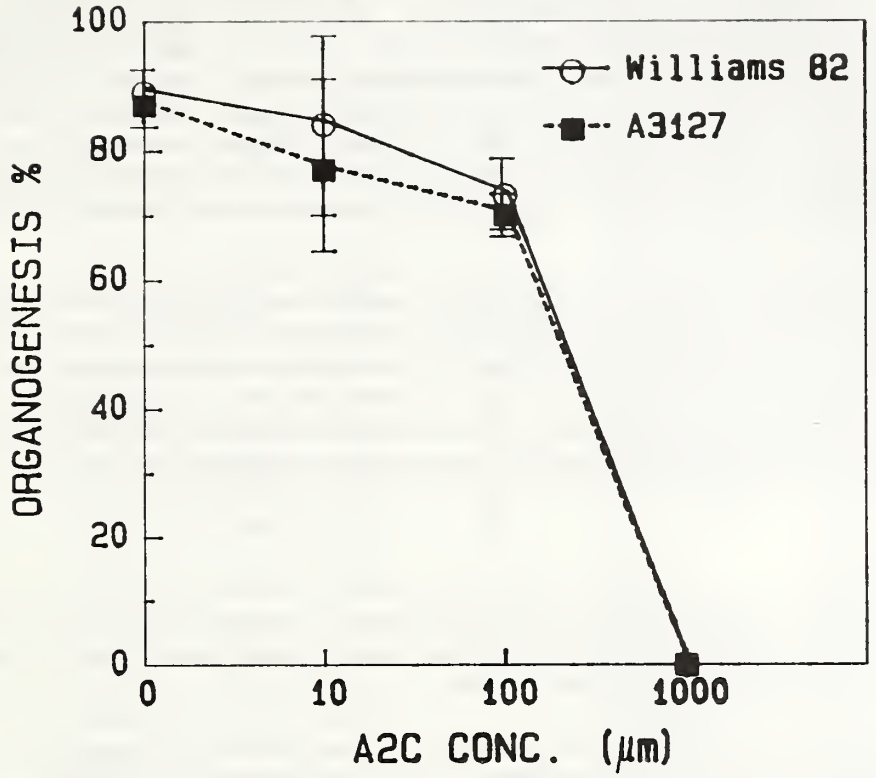

Figure 3. Effect of A2C on the formation of organogenic tissue cultures from Williams 82 and A3127 immature embryos.

herbicide resistance or methionine or proline overproduction in selected cultures and then regenerate plants that may also express these traits.

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4) Chromosome numbers of Pueraria phaseoloides and P. lobata.

The genus Pueraria DC. of Asian origin, is a member of the Glycine group of the subtribe Glycininae, tribe Phaseoleae. Tropical kudzu P. phaseoloides (Roxb.) Benth. and kudzu P. lobata (Willd.) Ohwi (van der Maesen, 1985), the two cultivated species in the genus, are used for forage, fodder, and soil conservation purposes in tropical and subtropical zones. However, none of the species of Pueraria are true domesticates. Lackey (1977) reported that the genus Pueraria has a close taxonomic affiliation with the genus Glycine. Perhaps, in the future, the species of Pueraria might constitute the tertiary gene pool for the soybean.

Attempts at establishing the chromosome number of Pueraria have been sporadic and contradictory with reports of a 2n number of 20, 22, and 24 (Goldblatt, 1981). We analyzed the somatic chromosome number from root tip squashes (Kumar and Hymowitz, 1988) of 27 accessions of P. phaseoloides and 7 accessions of P. lobata representing a diverse geographical origin (Table 1). We conclude that the somatic chromosome number of P. phaseoloides and P. lobata is 22.

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Table 1. Origin and chromosome number (2n) of Pueraria species

CU No.	Species	2n	Origin	Seed source
6	*Pueraria phaseoloides*	22	Sri Lanka	Pretoria, SA[1]
7	*Pueraria phaseoloides*	22	Singapore	Pretoria, SA[1]
8	*Pueraria phaseoloides*	22	Singapore	Pretoria, SA[1]
10	*Pueraria phaseoloides*	22	Taiwan	Pretoria, SA[1]
12	*Pueraria phaseoloides*	22	Australia	Q 1029[2]
13	*Pueraria phaseoloides*	22	Tanzania	Nairobi, Kenya[3]
15	*Pueraria phaseoloides*	22	?	CQ 367[4]
17	*Pueraria phaseoloides*	22	Rhodesia	CpI 26461[4]
18	*Pueraria phaseoloides*	22	Venezuela	CpI 28113[4]
20	*Pueraria phaseoloides*	22	Malaya	CpI 30028[4]
21	*Pueraria phaseoloides*	22	Sri Lanka	CpI 30135[4]
22	*Pueraria phaseoloides*	22	Sri Lanka	CpI 30136
29	*Pueraria phaseoloides*	22	Philippines	Los Banos, Phil.[5]
32	*Pueraria phaseoloides*	22	?	# 17263[6]
48	*Pueraria phaseoloides*	22	Brazil	CIAT 815[7]
52	*Pueraria phaseoloides*	22	Brazil	CIAT 7978[7]
54	*Pueraria phaseoloides*	22	Brazil	CIAT 8042[7]
57	*Pueraria phaseoloides*	22	Brazil	CIAT 8352[7]
67	*Pueraria phaseoloides*	22	Thailand	CIAT 17281[7]
81	*Pueraria phaseoloides*	22	Thailand	CIAT 17298[7]
82	*Pueraria phaseoloides*	22	Thailand	CIAT 17300[7]
83	*Pueraria phaseoloides*	22	Thailand	CIAT 17301[7]
85	*Pueraria phaseoloides*	22	Thailand	CIAT 17305[7]
86	*Pueraria phaseoloides*	22	Thailand	CIAT 17307[7]
113	*Pueraria phaseoloides*	22	Thailand	CIAT 18380[7]
114	*Pueraria phaseoloides*	22	Indonesia	KLM 950[8]
119	*Pueraria phaseoloides*	22	Indonesia	KLM 976[8]
2	*Pueraria lobata*		Korea	PI 326583[9]
30	*Pueraria lobata*	22	?	# 14536[6]
33	*Pueraria lobata*	22	?	# 17321[6]
35	*Pueraria lobata*	22	?	# 48977[6]
36	*Pueraria lobata*	22	Japan	?
38	*Pueraria lobata*	22	Japan	?
45	*Pueraria lobata*	22	?	Park Seed Co., N.C.

[1] Division of Plant and Seed Control, Pretoria, South Africa.
[2] Division of Plant Industry, CSIRO, Canberra, ACT, Australia.
[3] Division of Plant Quarantine, East African Agriculture, Nairobi, Kenya.
[4] Department of Primary Industries, Division of Agriculture, Brisbane, Australia.
[5] Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Banos, Philippines.
[6] Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois.
[7] CIAT, Cali, Columbia.
[8] Botanic Gardens of Indonesia, Bogor, Indonesia. Collected by K. L. Mehra for IBPGR.
[9] Southern Regional Plant Introduction Station, Experiment, Georgia

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5) 345 Updated information on the biosystematics of the Genus Glycine.

Table 1 contains the latest version of the species in the genus Glycine, authority, 3 letter code, 2n chromosome number, tentative genome designation, and the standards used in the determination of genomic affinities. Both the University of Illinois (IL) and the USDA (PI) numbers for each standard are presented.

Glycine clandestina intermediate (A_1A_1) and long pod (A_2A_2) forms have been combined into G. clandestina (A_1A_1). Glycine argyrea previously given the genome symbol A_3A_3 is now A_2A_2.

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Table 1. The species in the genus Glycine, three letter code, somatic chromosome number, genome symbols, and standard accessions.

Species	Code	2n	Genome symbol	Standards* IL	Standards* PI
Genus Glycine Willd.					
Subgenus Glycine					
1. G. arenaria Tind.	ARE	40	--		
2. G. argyrea Tind.	ARG	40	A_2A_2	768	505151
3. G. canescens F. J. Herm.	CAN	40	AA	401	440928
4. G. clandestina Wendl.	CLA	40	A_1A_1	425	440948
5. G. curvata Tind.	CUR	40	--		
6. G. cyrtoloba Tind.	CYR	40	CC	481	440963
7. G. falcata Benth.	FAL	40	FF	674	505179
8. G. latifolia (Benth.) Newell and Hymowitz	LAT	40	B_1B_1	373	378709
9. G. latrobeana (Meissn.) Benth.	LTR	40	--		
10. G. microphylla (Benth.) Tind.	MIC	40	BB	449	440956
11. G. tabacina (Labill.)	TAB	40	B_2B_2	370	373990
No adventitious roots		80	AAB_2B_2	506	440996
With adventitious roots		80	BBB_2B_2	640	483204
12. G. tomentella Hayata	TOM	38	EE	398	440998
		40	DD	614	446993
		78	?AAEE	363	339657
		80	AADD	485	441005
Subgenus Soja (Moench.) F. J. Herm.					
13. G. soja Sieb. and Zucc.	SOJ	40	GG		
14. G. max (L.) Merr.	MAX	40	GG		

*IL numbers are temporary University of Illinois accession numbers; PI numbers are permanent numbers assigned by the USDA.

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1) Comparative leaf morphology of soybean genotypes resistant and susceptible to Mexican bean beetle defoliation.//

The leaf morphology of soybeans resistant and susceptible to Mexican Bean Beetle (Epilachna varivestis Mulsant) are being compared by using scanning electron microscopy techniques. Ten resistant and nine susceptible genotypes from maturity groups IV through VIII have been cultivated under greenhouse and field conditions. Upper epidermal, lower epidermal, and cross-sectional morphology are being examined. Scanning electron microscopy preparative techniques include fixation of fresh tissues in 2.5% glutaraldehyde (pH. 7.0, 0.1 M phosphate buffer, 4 h, 4° C); phosphate buffer rinse (6 x 10 min/change, 4° C); fixation in 1% OsO_4 (pH7.0, 0.1 M phosphate buffer, 4 h, 4° C); distilled water rinse (6 x 10 min/change, 20° C); ethanol series dehydration (20, 40, 60, 80, 100% x 3, 30 min/change); ethanol cryofracture (Humphreys et al., 1974) and critical-point drying (Anderson, 1951).

Greenhouse-cultivated genotypes demonstrated some variation in epicuticular wax deposition. Figures 1 through 3 demonstrate the differences observed in the epicuticular wax found on the upper epidermal surface of resistant and susceptible genotypes. One Mexican bean beetle-resistant genotype lacked epicuticular wax deposition (Fig. 1, PI 229358). The resistant genotype PI 417124 (Fig. 2) showed heavy deposition of 'fine' textured epicuticular wax in contrast to the susceptible genotype PI 201428 (Fig. 3) which had a 'coarse' texture deposition. We do not suggest that these variations are related to Mexican bean beetle resistance. Further investigations are required to determine the significance of these results.

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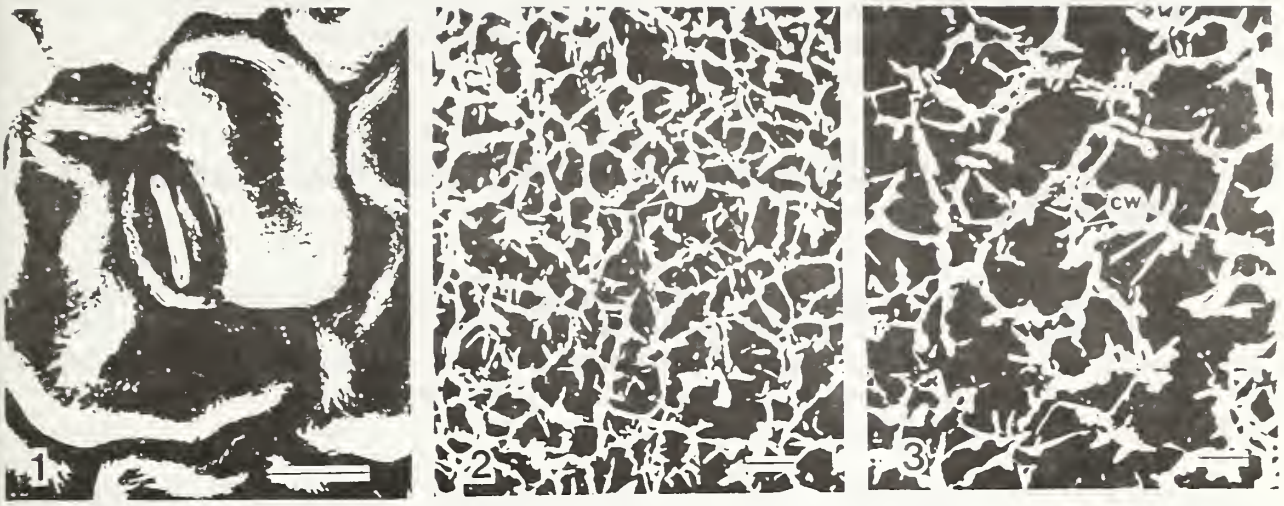

Figure 1. Upper epidermal surface of PI 229358. Bar = 10 microns.

Figure 2. Upper epidermal surface of PI 417124, fine epicuticular wax (fw). Bar = 1 micron.

Figure 3. Upper epidermal surface of PI 201428, coarse epicuticular wax (cw). Bar = 1 micron.

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1) Effect of ozone-stressed soybean foliage on the fecundity of the Mexican bean bettle.

Mexican bean beetle (Epilachna varivestis Mulsant) (MBB) adults prefer to feed on soybeans that have been stressed by ozone (Chappelka et al., 1987). Antibiosis, another form of plant resistance to insect defoliation, may also be affected by ozone stress. One aspect of antibiosis, fecundity, was examined in the following experiment.

Two soybean cultivars, 'Forrest' and 'Essex', were exposed to incremental levels of ozone in open-top field chambers. Treatments consisted of chambers receiving carbon-filtered air (CF) and nonfiltered air supplemented with 0.000, 0.03, or 0.06 ppm O_3 (NF, NF+3, and NF+6, respectively). The cultivars were planted in 10-inch pots, 3 per pot, and grown to the V2 stage before being transferred to the open-top chambers. After 13 days of treatment (h/d) the pots were removed to a greenhouse and placed in insect cages (8 pots/cage). Each cage (2'x3'x4') contained 32 adult MBBs, collected from the field. Counts of eggs, egg masses, and living beetles were made after 6 days.

Data were analyzed by ANOVA and means were separated by Duncan's Multiple Range Test. There was no significant difference between the mean number of eggs per egg mass with any treatment. This supports the findings of Kitayama et al. (1979) and Brooks (1986) who reported that stress-induced fecundity changes in the MBB, except water stress, were reflected in a reduced number of egg masses rather than a reduced number of eggs/mass. Thus, only one set of egg-count data is presented herein, i.e., total eggs layed (Table 1).

Almost three times as many eggs were layed by MBBs in cages containing the Forrest cultivar, compared with the Essex cultivar, averaging 361 vs 126 eggs per cage, respectively. The reason for this cultivar difference is not understood. The greatest number of eggs per cage, over 600, was found in the NF+6 treatment of the Forrest cultivar. This treatment had a significantly ($P<0.05$) greater number of eggs than any other treatment for either cultivar. The second highest average egg count (325) was found with the NF+3 treatment of the Forrest cultivar.

A similar increase in MBB fecundity with ozone-exposed foliage was not found with the Essex cultivar. Based on visible injury, Essex is among the most tolerant and Forrest among the most sensitive of soybean cultivars to ozone (Heagle and Letchworth, 1982). However, Chappelka et al. (1987) found increased preference for ozonated foliage with both the Forrest and Essex cultivars, even though Essex showed much less visible damage from ozone. If the results of future fecundity studies are similar to those reported herein it could indicate that different plant defense mechanisms are responsible for preference and antibiosis effects.

Table 1. Mean number of eggs layed and mean number of surviving MBB adults per cage after 6 days feeding by MBBs on two soybean cultivars exposed to different ozone treatments (3 replicates).

Cultivar	Treatment	Ave. ozone[1]	Eggs[2]	Survival[3]
Forrest	CF	0.0339	290 bc	22.3 a
Forrest	NF	0.0498	225 bc	16.3 a
Forrest	NF+3	0.0900	325 b	20.3 a
Forrest	NF+6	0.1277	604 a	22.0 a
Essex	CF	0.0298	246 bc	14.3 a
Essex	NF	0.0494	71 bc	12.0 a
Essex	NF+3	0.0892	148 bc	15.7 a
Essex	NF+6	0.1252	40 c	13.7 a

1) Average ozone concentration over 13-day (7h/d) plant exposure period, expressed in ppm. Ambient air averaged 0.0568 ppm ozone during this period.

2) Means followed by the same letter are not significantly different (5% level, Duncan's multiple range test).

3) Each cage initially contained 32 MBBs.

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1) Field screening for inefficient Bradyrhizobium japonicum strain exclusion.

Strains of Bradyrhizobium japonicum capable of forming a high proportion of soybean nodules may not always be the most efficient in dinitrogen fixation. Highly competitive but inefficient strains present in many nodules after growth of plants in soil containing a mixture of strains may reduce nodule mass, nitrogenase activity, and seed yield (Caldwell and Vest, 1970; Singleton and Stockinger, 1983). While nodule occupancy by different strains is influenced by factors such as seasonal environment (Hunt et al., 1985), evidence of host plant genetic control exists (Caldwell and Vest, 1968; Cregan and Keyser, 1986). Howle et al. (1987) studied six group VII cultivars for specificity of nodulation with the efficient bradyrhizobial serogroup 110 but were unable to detect genetic differences in recovery. The objective of our study was to identify genotypes from a small group of soybean Plant Introductions (PI) in maturity groups V to X for possible exclusion of inefficient bradyrhizobial serogroups under South Carolina field conditions.

Materials and methods: Ten seeds of each of 454 PI genotypes and six released cultivars were placed in hills, spaced on 1 m centers, on June 3, 1986 at Blackville, SC. The experimental design was a randomized complete block with two replications. Soil type was a Dothan loamy sand (Plinthic Paleudults). Overhead irrigation was provided as necessary to prevent water stress. Hills were excavated as the plants reached the R1 to R3 growth stage and nodules were frozen in plastic bags or glass vials. Nodule occupancy by serogroups USDA 24, 46, 94, and 122 of B. japonicum was determined in 24 nodules from each genotype by antibody-agglutination (Means et al., 1964).

Results and discussion: Genotypes with low and high percentages of each serogroup were identified (Table 1). Only 382 of the 454 PI's planted survived to sampling. Serogroups 24 and 122 produced greater than 29% of the nodules on more genotypes than serogroups 46 and 94, indicating that these strains differ in competitiveness or that the genotypes used differ in their exclusion abilities. Serogroup 122, the most efficient in dinitrogen fixation of those reported, was completely excluded by only 18 genotypes. Further competition and genetic studies need to be done for confirmation of these results. However, these initial data indicate that desirable germplasm may exist for incorporation into a breeding program to increase efficient nodulation in Coastal Plain soils.

Five of the most promising genotypes are listed in Table 2. The selection criterion used to identify these genotypes was 4% or less of the nodules formed by each inefficient serogroup (24, 46, and 94) and greater than 33% by the more efficient serogroup 122. These genotypes nodulated with a much more desirable proportion of serogroup 122 than the cultivars 'Dyer', 'Centennial', and 'Braxton'.

Table 1. Numbers of genotypes of soybean with low and high percentages of nodules in four Bradyrhizobium japonicum serogroups, field test, 1986.

	serogroup				
Nodules	24	46	94	122	No reaction
--%+-	-------- No. of genotypes----------				
> 29	25	2	1	41	269
21-25	37	6	7	76	59
13-17	55	22	13	151	42
4- 8	98	83	36	96	12
0	167	269	325	18	0

+ Based on 24 nodules tested from each genotype.

Table 2. Nodule occupancy of 5 selected soybean genotypes and 3 released cultivars by 4 Bradyrhizobium japonicum serogroups, field test, 1986.

Genotype	Maturity group	serogroup				No reaction
		24	46	94	122	
		---% nodule occupancy+--				%
PI 88820	V	0	0	0	33	46
PI 407765	V	4	0	0	33	29
Dyer	V	13	0	0	0	25
PI 209908	VI	0	0	0	33	33
Centennial	VI	8	0	0	0	50
PI 165675	VII	0	0	0	42	17
Braxton	VII	0	0	0	0	71
PI 307837	X	0	4	0	33	17

+Based on 24 nodules tested from each genotype. Totals do not = 100% because of the presence of nodules formed by serogroups not reported.

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2) Differential nodulation of soybean cultivars in the presence of Hoplolaimus columbus.

Columbia lance nematode, CLN, [Hoplolaimus columbus Sher] is a serious endo-ectoparasite of soybean and causes substantial yield reductions in the Southeastern coastal plain. The importance of genetic tolerance and cultural practices as CLN management factors has become more apparent since EPA deregistration of previously utilized fumigant nematicides. Resistance based upon reduced infection levels of CLN in soybean has not been found. Soybean cultivars tolerant to CLN have been identified; however, many of them still sustain substantial seed yield losses (Nyczepir and Lewis, 1979). Boerma and Hussey (1984) demonstrated that 'Centennial', 'Coker 156', and 'Wright' exhibited greater tolerance than 'Bragg' or 'Coker 237'. In evaluations of cultivars and nematicides in CLN-infested fields, 'Braxton' has shown to be extremely susceptible whereas 'Foster' and 'Coker 368' have yielded equal to or greater than Centennial (Mueller et al., 1986; Mueller and Sanders, 1987). Limited information is currently available regarding the inheritance or mechanisms of CLN tolerance in soybean (Maxwell et al., 1971; Weiser et al., 1987). Yield losses due to CLN are, however, partially alleviated by in-furrow subsoiling, possibly because of greater early season tap root growth (Blackmon and Musen, 1974).

Sedentary endoparasites such as soybean cyst nematode [Heterodera glycines Ichinohe] suppress soybean nodulation (Ko et al., 1984), and decreases root lectin binding of Bradyrhizobium japonicum (Huang et al., 1984). Appel and Lewis (1984) observed a similar reduction in nodulation with increasing CLN density on a susceptible cultivar, 'Davis'. The objective of this study was to determine the relationship of nodulation and tolerance in CLN infected soybean.

Materials and methods: Plots of Foster, Centennial, Coker 368, and Braxton were planted with and without 0.3 m deep in-furrow subsoiling in rows on 0.95 m centers in a field of Varina sandy loam soil (clayey, kaolintic, thermic Plinthic Paleudults) near Blackville, SC, on 17 July 1986. The mean infestation level of CLN at planting was 120 CLN per 100 cm^3 of soil. Bradyrhizobium japonicum was not added as inoculum; therefore, all nodules formed contained endogenous strains. Surveys of endogenous strains conducted in this geographic region have shown a mixture of seven serologically identifiable components (Caldwell and Hartwig, 1970).

Five single plants per cultivar, grown with and without subsoiling, were excavated at random within predetermined sampling areas, 1-m long, within the plots at the V3, V5, R1, and R3 plant growth stages. For each plant, roots were rinsed in water, nodules removed and counted, and the roots weighed and placed in a mist chamber for determination of CLN per g fresh weight of root.

Results and discussion: Recovery of nodules at V3 was too low for meaningful comparisons. Braxton did not nodulate in the non-subsoiled plots, and in the subsoiled plots had significantly fewer nodules than the three tolerant cultivars at all sampling dates except R3. Coker 368 differed from the other two tolerant cultivars in that it had significantly fewer nodules in the subsoiled plots at V5, R1, and R3. At R3, Coker 368 had the lowest number of nodules of any other cultivar in the subsoiled plots; however, it had the greatest number of nodules in the non-subsoiled plots. Centennial and Foster continued to have a greater number of nodules than Braxton at R3, although they apparently slough nodules between R1 and R3 at a more rapid rate than Coker 368, which showed an increase in nodule number.

Table 1. Nodulation by tolerant and susceptible soybean cultivars in the presence of H. columbus under subsoiled and non-subsoiled conditions at each of four plant growth stages.

Cultivar	Average number of nodules at growth stage:[a] V3	V5	R1	R3
		subsoiled		
Centennial	4.4a	27.6a	23.4a	22.2a
Foster	0.2b	19.8b	23.6a	23.4a
Coker 368	2.0b	13.6c	2.8b	0.0c
Braxton	0.0b	1.2d	0.0c	7.0b
		non-subsoiled		
Centennial	0.0a	2.4c	14.6b	0.0c
Foster	1.4a	21.0a	19.6a	11.8b
Coker 368	1.8a	15.4b	14.8b	23.6a
Braxton	0.0a	0.0d	0.0c	0.0c

[a]Means in a column followed by the same letter are not significantly different (FLSD, P=0.05).

These results indicate that the high yield losses associated with CLN infection of Braxton may be related to low levels of nodulation. In numerous other studies, we have observed that CLN-infected Braxton has shown chlorotic symptoms typical of nitrogen deficiency. The ability to produce and sustain a relatively large number of nodules through the late vegetative and reproductive growth stages may be one component of the genetic mechanism(s) of CLN tolerance in Foster, Centennial, and Coker 368. Further studies to determine actual levels of dinitrogen fixation are needed to confirm this hypothesis.

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1) Evaluation of commercial soybean cultivars and advance breeding lines for non-preference to Heliothis zea.

Corn earworm (Heliothis zea) damage to soybean and corn has become very severe in recent years. Heavy infestation can cause complete crop loss if control measures are not applied (Turnipseed, 1973). This pest feeds on both the foliage and developing seeds in the pods. Soybeans do become primary host as corn (Zea mays) and cotton (Gossypium hirsutum) become mature and consequently less attractive for oviposition (Freeman et al., 1967). Each corn earworm larva is capable of damaging 6 to 8 pods and about 7 seeds between 4th and 6th instar (Boldt et al., 1975; Smith and Bass, 1972). At the present time in Maryland, corn-earworm-resistant varieties are not available to the farmers. The objective of this study was to determine the extent of susceptibility or non-preference to corn earworm among the recommended soybean varieties belonging to maturity groups IV and V, and some advance breeding lines bred for resistance to this insect.

Materials and methods: Seven commercial soybean varieties (Table 1) of maturity groups IV and V, and three advance breeding lines, D75-10169, D82-3298, and D85-904, were tested for non-preference in the screen house during the growing season in 1987. Experiment was planted on May 12, 1987 in a randomized block design with four replications. Each plot consisted of a single row, 10.7 m long, and the distance between rows being 0.76 m. During full bloom period on July 14, 1987, 4,000 corn earworm pupae were placed in the screen house (720 m^2). Two hundred and fifty pupae were placed at each of the four corners of each replication. By July 24, 1987, 90% of the pupae had emerged. Observations included egg count/plant, number of larvae/plant and % leaf damage. The upper four trifoliolate leaves of each plant were used for egg and larval count. The amount of leaf area damaged by corn earworm larvae is based on the upper-most 4 trifoliolate leaves and is expressed in percent.

Experimental results: Painter (1951) classified the phenomenon of insect resistance into three main components, i.e., non-preference for oviposition, food or shelter; antibiosis (adverse effect of plant on the biology of insect) and tolerance (repair, recovery, or ability to withstand infestation). Data concerning number of corn earworm eggs, larvae, and percent leaf damage per plant are given in Table 2.

Among the ten cultivars evaluated under screen house conditions, least egg laying activity was observed on advance breeding line D82-3298 (8.7 eggs/plant), followed by another advance breeding line, D85-904 (9.8 eggs/plant) and 'Douglas', a recommended soybean cultivar (10.2 eggs/plant). Regarding the number of corn earworm larvae, the least number of larvae was observed D85-904 (4.2 larvae/plant), followed by 'Spark', a commercial variety (5.5 larvae/plant) and D82-3298 (6.3 larvae/plant). It appears that two advance breeding lines, namely, D82-3298 and D85-904, exhibit high levels of non-preference to corn earworm whereas Spark had a very high number of eggs per plant but the larval count per plant was very low. This perhaps may be due to antibiosis.

Table 1. Source, maturity group and parentage of soybean cultivars evaluated for non-preference and tolerance to Heliothis zea.

Cultivar	Source	Maturity group	Parentage
Morgan	Maryland	IV	Union X Miles
Stafford	Virginia	IV	V66-318 X V68-2331
Regal	Indiana	IV	(Union X PI86972-1) PI84637
Union	Illinois	IV	Williams X SL11
Douglas	Kansas	IV	Williams X Calland
Spark	Kansas	IV	Williams X Calland
Essex	Virginia	V	Lee X SS-7075
D75-10169	Mississippi	V	(Bragg X PI 229.358) Goven
D82-3298	Mississippi	V	(Bedford X Forrest) D-75-10169
D85-904	Mississippi	V	(Bedford X Forrest) PI82.3233

Table 2. Mean egg count, larval number and percent leaf damage per plant by H. zea on certain soybean cultivars.

Cultivar	Eggs	Larvae	Leaf damage
Spark	29.1	5.5	33
D75-10169	26.7	8.0	38
Regal	10.6	9.7	42
D82-3298	8.7	6.3	44
Stafford	25.3	7.7	45
D85-904	9.8	4.2	46
Union	22.4	8.3	49
Morgan	40.3	9.6	50
Essex	15.8	7.4	51
Douglas	10.2	6.5	52

The amount of leaf damage is an indication of either antibiosis or tolerance or both. The data indicated Spark sustained minimum leaf loss (33%), followed by D75-10169 (38%) and Regal (42%). Although statistical analysis failed to show any significance at 0.05 probability level, there is some indication that the minimum leaf damage and minimum larvae/plant for Spark may be due to antibiosis or tolerance or both. Further studies will be conducted to verify these results.

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1) Genetic relationship among 'Forrest', 'Peking' and PI 90763 for resistance to Heterodera glycines Race 3.

Soybean cyst nematode (SCN) was first reported in the USA in 1954 by Winstead et al. (1955). Soybean production suffers great damage by SCN in several states. Peking, a selection from PI 17852, has been the source of resistance in breeding several SCN-resistant soybean cultivars including 'Forrest'. Genetic studies indicate resistance in Peking is conditioned by three independent recessive genes, rhg1, rhg2, rhg3 and a dominant gene, Rhg4, which is closely linked to the I locus (Caldwell et al., 1960; Matson and Williams, 1965). Caldwell et al. (1960) also reported lack of segregation among crosses between Peking and PI 90763 against the North Carolina SCN populations. This work was reported prior to the establishment of race nomenclature. Recently, in our lab we observed similar results for SCN race 3 reaction (unpublished results). However, the genetic relationship between Forrest x Peking, and Forrest x PI 90763 for SCN race 3 reaction has not been documented. The purpose of this report is to provide additional evidence to show that Peking and PI 90763 have the same loci conditioning resistance to SCN race 3 reaction.

Materials and methods: The soybean cultivar Forrest was crossed with Peking and PI 90763 during summer of 1983. All of the parental genotypes are resistant to SCN race 3. Both Peking and PI 90763 have a black seed coat and are in maturity group IV. Forrest has a yellow seed coat and is in maturity group V. The F_1 plants were grown in the winter nursery in Puerto Rico to obtain the F_2 population and these plants were advanced to generate F_3 families at the Delta Center of the University of Missouri, Portageville. The F_1 and F_2 soybean plants were grown in SCN-free soil to produce seed for this study. Evaluations in the greenhouse included 157 F_2 plants and 102 randomly selected F_3 families for the cross Forrest x PI 90763 and 50 F_2 plants for the cross Forrest x Peking. The F_3 families for Forrest x Peking were not available. The techniques of evaluation were already described (Rao-Arelli and Anand, 1986, 1987). The index of parasitism (IP) for each plant was calculated as (number of white females on a given plant/mean number of white females on susceptible check Essex x 100). An IP of 10% or more was classified as susceptible, whereas an IP of less than 10% was classified as resistant (Golden et al., 1970). Based on the reaction of individual plants, the F_2 plants were classified resistant or susceptible and the F_3 families were classified resistant, segregating or susceptible.

Results and discussion: The F_2 population from the cross Forrest x Peking did not segregate for resistance. Similarly, Forrest x PI 90763 did not segregate for resistance in either F_2 or F_3 generation (Table 1).

Table 1. Reaction of soybean parents, F_2 plants, F_3 families, host differentials and susceptible check Essex to SCN race 3.

Entry	Number of Plants		Mean number of white females	Range of white females
	Resistant	Susceptible		
Essex	0	10	165	124-217
Peking	10	0	1	0-3
PI 90763	10	0	1	0-2
PI 88788	10	0	2	0-6
Forrest	10	0	6	0-9
Forrest x Peking (F_2)	50	0	1	0-8
Forrest x PI 90763(F_2)	157	0	1.6	0-14
Forrest x PI 90763(F_3)	102	0	0.1	0-16

Peking is the source of resistance in cv. Forrest for SCN race 3 reaction. It has been observed that there was no segregation between Peking x PI 90763 for SCN race 3 (unpublished results). The lack of segregation strongly indicates that the same alleles condition resistance in Peking, PI 90763 and cv. Forrest for SCN race 3 reaction.

However, PI 90763 has a range of 0-2 white females and a mean number of one/plant roots when evaluated for SCN race 3 reaction. Forrest has a slightly higher range of 0-9 white females with a mean of six white females/plant roots. Perhaps difference of minor genes might be contributing for unequal number of white females and it is presumed that Forrest lacks these minor genes for conditioning resistance to SCN race 3.

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1) Inheritance of reaction to strains G_5 and G_6 of soybean mosaic virus (SMV) in differential soybean cultivars.

Introduction: Soybean mosaic virus (SMV) causes different symptoms depending upon the combination of soybean genotype and virus strain. Cho and Goodman (1979) classified various SMV isolates from soybean germplasm into seven (G_1-G_7) strain groups based on the reactions of a set of differential soybean cultivars. G_5 and G_6 are moderately virulent strains that cause mosaic symptoms in 'Lee 68' and 'York' and necrosis in 'Kwanggyo'. The two strains are differentiated by reactions of cultivar 'Marshall' which is resistant to G_5 but necrotic to G_6. The other two differential groups, represented by PI 96983 and 'Ogden', are both resistant to strains G_5 and G_6. SMV-G_5 and G_6 strains were chosen for our genetic study because the genetics of reaction to these strains has not been well studied. Kiihl and Hartwig (1979) reported that PI 96983 carries a dominant gene labeled Rsv for resistance. Buzzell and Tu (1984) demonstrated that the resistance from cultivar 'Raiden' is controlled by another gene symbolized Rsv_2 which segregated independently of Rsv. Genes in other differential cultivars have not been investigated. The objectives of this study were to further characterize the inheritance of reaction to SMV-G_6 and to determine the inheritance patterns of resistance to SMV-G_5 in differential soybean cultivars.

Materials and methods: Crosses were made in the greenhouse at Blacksburg. F_1 plants were grown in the field at Warsaw without inoculation with SMV. F_2 populations were screened with SMV-G_5 and G_6 in the greenhouse during the winter using supplementary light. Each of the F_2 populations was grown in a metal flat. In each flat three rows of F_2 seeds (20-25 seeds/row) were planted. A row of both parents of each cross and a row of Lee 68 were also planted in each flat as resistant and susceptible checks, respectively. Two pots (6-10 plants/pot) of each differential cultivar were included in the test for strain identification. Inoculum of each strain was prepared by grinding infected leaves in 0.01 M sodium phosphate buffer (approx. 10 ml per g leaf tissue) at pH 7 with a mortar and pestle. Inoculations were made by gently rubbing the inoculum with a pestle onto both fully expanded unifoliolate leaves of each seedling (about 2 weeks of age) lightly dusted with carborundum powder. The inoculated plants were examined for symptoms of infection in two-week intervals after inoculation until blooming. Plants of each F_2 population were classified as no symptom (R), systemic mottling (S), and systemic necrosis (N). The necrotic plants were included with resistant plants for testing genetic ratios as Kiihl and Hartwig (1979) proposed. Chi-square tests were made for goodness of fit to the proposed segregation ratio.

Results and discussion: The distribution of F_2 plants for reaction to SMV-G_5 from eight crosses is presented in Table 1. The data from R x S crosses showed satisfactory fits to expected ratios for simple

Table 1. Seedling reaction to F_2 populations from crosses between representative soybean cultivars when inoculated with SMV-G_5.

Crosses	R	N	S	Ratios	X^2(3:1)	P
Ogden x Lee 68 (RxS)	19	43	25	R+N:S=62:25	0.648	.30-.50
Ogden	16	0	0			
Lee 68	0	0	15			
York x Marshall (SxR)	12	29	18	R+N:S=41:18	0.955	.30-.50
York	0	0	13			
Marshall	5	0	0			
York x Ogden (SxR)	24	51	22	R+N:S=75:22	0.278	.50-.70
York	0	0	16			
Ogden	21	0	0			
Ogden x Kwanggyo (RxN)	33	57	0	N:R=57:33	6.533	.01-.02
Ogden	28	3	0			
Kwanggyo	3	15	0			
Kwanggyo x Marshall (NxR)	14	8	0	R:N=14:8	1.174	.20-.30
Kwanggyo	3	8	0			
Marshall	5	0	0			
York x Kwanggyo (SxN)	3	18	5	R+N:S=21:5	0.461	.30-.50
York	0	0	15			
Kwanggyo	1	6	0			
Ogden x Marshall (RxR)	17	3	0	R+N:S=20:0	-	-
Ogden	16	2	0			
Marshall	6	0	0			
York x Lee 68 (SxS)	0	0	26	R+N:S=0:26	-	-
York	0	0	9			
Lee 68	0	0	7			

Table 2. Segregation of F_2 populations for reaction to SMV-G_6.

Crosses	R	N	S	Ratios	X^2(3:1)	P
Ogden x Lee 69 (RxS)	3	39	15	R+N:S=42:15	0.053	.70-.80
Ogden	8	1	0			
Lee 68	0	0	11			
PI 96983 x Lee 68 (RxS)	8	10	6	R+N:S=18:6	0.000	> .99
PI 96983	6	0	0			
Lee 68	0	0	13			
PI 96983 x York (RxS)	5	7	4	R+N:S=12:4	0.000	> .99
PI 96983	5	0	0			
York	0	0	7			
York x Ogden (SxR)	9	39	14	R+N:S=48:14	0.194	.50-.70
York	2	0	20			
Ogden	25	5	0			
York x PI 96983 (SxR)	17	21	14	R+N:S=38:14	0.103	.70-.80
York	0	0	13			
PI 96983	2	0	0			
York x Marshall (SxN)	5	21	13	R+N:S=26:13	1.444	.20-.30
York	0	0	4			
Marshall	2	2	0			
York x Kwanggyo (SxN)	3	27	10	R+N:S=30:10	0.000	> .99
York	0	0	10			
Kwanggyo	2	4	0			
Ogden x Kwanggyo (RxN)	27	56	0	N:R=56:27	2.510	.10-.20
Ogden	28	2	0			
Kwanggyo	2	8	0			
Odgen x Marshall (RxN)	71	20	0	R:N=71:20	0.443	.50-.70
Ogden	12	2	0			
Marshall	0	2	0			
PI 96983 x Ogden (RxR)	21	0	0	R+N:S=21:0	-	-
PI 96983	7	0	0			
Ogden	11	0	0			

inheritance. This agrees with previous inoculations of crosses with Ogden and Marshall times a susceptible tester in which SMV strain G1 was used. The results from the cross between Ogden and Marshall exhibited no segregation of susceptibility, suggesting that the resistance genes in Ogden and Marshall either share a common locus or are closely linked. The S x N cross, York x Kwanggyo, gave a good fit to 3:1 ratio with necrosis dominant. No resistant or necrotic plants were observed in the F_2 population from the S x S cross. The R x N crosses produced no susceptible plants but gave different segregation patterns of resistance vs. necrosis. The necrotic reaction in Kwanggyo appears dominant to the resistance in Ogden but recessive to the resistance allele in Marshall. However, the data from the York x Kwanggyo cross cannot be considered conclusive because of the small sample. Also, there appears to be some deficiency of N plants in the Ogden x Kwanggyo cross.

Similar results were obtained from F_2 populations inoculated with SMV-G_6 (Table 2). All R x S crosses segregated monogenically with resistance dominant. It seemed that PI 96983 and Ogden carry allelic genes for resistance to SMV-G_6 since the cross between them did not segregate for susceptibility. The two S x N crosses involving Marshall and Kwanggyo as necrotic parents gave good fits to 3:1 segregation with necrosis dominant to susceptibility. However, in the R x N crosses, the resistance allele in Ogden seems to be dominant to the allele in Marshall but recessive to the allele in Kwanggyo for necrotic reaction.

The necrotic plants in the F_2 populations from R x S and R x R crosses were considered resistant in X^2 tests because no virus was detected in them by ELISA (Lister, 1978). The R x S crosses inoculated with either SMV-G_5 or G_6 segregated 1R:2N:1S. This supports the hypothesis that the necrotic plants are heterozygous. The N x R crosses did not give consistent segregation patterns with regard to dominance. Further tests are needed to clarify this point. In the F_2 populations from S x N crosses, a few resistant plants were observed. The reason for this unexpected segregation is not clear at this point. A possible explanation would be that the necrotic parents (Marshall and Kwanggyo) in the S x N crosses did not give uniform response to the viral strains (occasional resistant reactions were seen), thus some resistant plants were found in their progenies. Also, the cross of Ogden x Marshall (RxR) produced three necrotic plants in the F_2 population. This is probably because Ogden occasionally produces necrotic plants when inoculated with SMV-G_5 or G_6. The mixed reactions of Ogden and Kwanggyo indicate that the necrotic reaction might not be completely under genetic control. We are continuing to evaluate the reaction of the complete set of crosses among these parents for reaction to SMV-G_6.

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2) Genetics of reaction to type strain (G_1) of soybean mosaic virus (SMV) in six differential soybean cultivars.

Introduction: Inheritance of reaction to SMV in soybeans has been studied by various workers using different SMV strains. Kiihl and Hartwig (1979) first detected two allelic genes symbolized Rsv and rsv^t in resistant cultivars PI 96983 and 'Ogden', respectively. The SMV strains used in their study were identified as G_2 and G_3 (Cho and Goodman, 1979). Buzzell and Tu (1984) found a different gene (Rsv_2) for resistance to SMV-G_7 and G_7A in cultivar 'Raiden', and demonstrated that this gene segregated independently of the Rsv gene in PI 96983 when tested with SMV-G_6. Lim (1984) reported that 'Suweon 97' and PI 486355 carry single dominant genes at two additional independent loci for resistance to SMV-G_2, G_7, or C14.

We have been investigating the genetic relationships among six differential cultivars (equivalent to Cho and Goodman's indicator hosts) using the type strain G_1 of SMV. In a previous report (Buss et al., 1987), we included some of our preliminary results from a set of allelism tests that did not include the crosses of Ogden x York, Ogden x Marshall, and Ogden x Kwanggyo. We report here the data obtained from a 1987 field inoculation of F_3 lines from these three crosses along with some repeated tests of previous crosses. In addition, F_2 populations were grown and inoculated in the greenhouse to compare the effects of two environments on the expression of the disease.

Materials and methods: The field evaluation for reaction to SMV-G_1 of F_3 lines from crosses among the differential cultivars was conducted as previously (Buss et al., 1987). Leaf samples for ELISA (Lister, 1978) were taken from F_3 rows containing only one or two symptomatic or necrotic plants. The SMV-G_1 strain identity was verified by inoculation the differential cultivars in the field. The screening method for reaction of F_2 populations to SMV-G_1 was similar to that used for SMV-G_5 and G_6 (Chen et al., 1988).

Results and discussion: The F_2 data (Table 1) from the two resistant cultivars crossed with susceptible 'Lee 68' showed good fits to the expected 3:1 ratio with resistance dominant, indicating that York and Ogden each possess single genes for resistance. The lack of segregation for susceptibility in the F_2 (Table 1) and F_3 (Table 2) from crosses among resistant cultivars furnished evidence that the resistance genes in all the resistant parents are alleles at a single locus. Although occasional symptomatic plants were detected in F_3 rows from five R x R crosses (Table 2), most of them gave negative reactions in ELISA. Thus, we felt confident that these symptomatic plants did not represent genetic segregation.

The results of this study and previous investigations indicate that the five resistant cultivars carry single dominant genes at a common locus for resistance to SMV-G_1. Although the possibility of linkage can not be ruled out, it is very unlikely that five separate genes from different sources are closely linked.

Table 1. Seedling reaction of F_2 populations from crosses between differential soybean cultivars when inoculated with SMV-G_1.

Crosses	Cross type	No. of plants			X^2(3:1)	P
		R	N	S		
York x Lee 68	(RxS)	46	32	27	0.051	.75-.90
Ogden x Lee 68	(RxS)	58	34	26	0.553	.25-.50
York x Ogden	(RxR)	94	5	0	-	-
York x Marshall	(RxR)	66	17	0	-	-
York x Kwanggyo	(RxR)	44	19	0	-	-
Ogden x Marshall	(RxR)	39	0	0	-	-
Ogden x Kwanggyo	(RxR)	81	5	0	-	-
Kwanggyo x Marshall	(RxR)	30	6	0	-	-

Table 2. Seedling reaction of F_3 lines from crosses among resistant soybean cultivars to inoculation with SMV-G_1.

Crosses	No. of F_3 rows			No. of Plants		
	Total	Homo-R	Seg.	S	N	Neg.#
York x Ogden	158	151	7[+]	17	3	9
Ogden x Marshall	131	122	9[++]	10	9	19
Ogden x Kwanggyo	114	104	10[++]	14	13	23
York x Kwanggyo	71	52	19[++]	32	23	55
Kwanggyo x Marshall	71	68	3[++]	4	9	13

[+] only 1 row fits 3R:1S; all others had 1 or 2 S plants.

[++] all rows had only 1 or 2 S plants.

[#] negative ELISA reaction for SMV.

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3) Introgression of a gene for resistance to soybean mosaic virus (SMV).

We have successfully transferred a gene for SMV resistance to a high-yielding, susceptible soybean cultivar, 'Essex', using the backcross method. The resistant donor parent used in the initial cross was 'Epps' which acquired its resistance to SMV from PI 96983. The pedigree of Epps is (Pickett 71 (2) x [Dare (2) x PI 96983] x J74-47). PI 96983 has been demonstrated to carry a single dominant gene, Rsv, for resistance to SMV strains G_1 through G_6. It is susceptible to G_7A and necrotic to G_7. 'Pickett', 'Dare', and J74-47 are susceptible to all the known strains of SMV.

The reaction of Epps to SMV was evaluated only with the type strain G_1 before the backcrossing program was initiated. The cross of Essex x Epps was made in the field in 1980 at the Eastern Virginia Agricultural Experiment Station, Warsaw. F_1 plants were grown in the field at Warsaw in 1981 without SMV inoculation and were backcrossed to Essex. The subsequent backcrosses were conducted in the greenhouse at Blacksburg. The progenies from each backcrossing generation were grown in 10-in. plastic pots and hand-inoculated with SMV-G_1. The BC_5 plants were evaluated for reaction to SMV-G_1 and the susceptible plants were discarded. Seeds were harvested separately from the resistant plants and were progeny-tested for reaction to SMV-G_1. The segregating progenies were discarded. Several homozygous resistant progenies that appeared to be most like Essex were harvested in bulk for field testing. One of these line, V85-5344, was evaluated for reaction to differential SMV strains in the greenhouse. Reactions of V85-5344 and its ancestors to eight SMV strains are shown in Table 1. It is obvious that the original reaction of the gene as found in PI 96983 was transmitted intact through 11 crossing generations.

When our allelism tests with the other differential cultivars began to show that many of their resistance genes were allelic (see previous article), we were concerned that the differential reactions might be due to some genetic or cytoplasmic modifiers of the Rsv gene. The fact that the specific reaction of the gene from PI 96983 was not modified through eleven generations, with no selection for reaction to strains G_7 or G_7A, is evidence that the reaction of Rsv is transmitted intact as a single unit. V85-5344 was evaluated in the field in 1986 and 1987 for agronomic performance and no alteration of important agronomic traits was observed in comparison to Essex.

Table 1. Reactions of isoline V85-5344 and its related parents to differential SMV strains.

Entry	SMV strain							
	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_7A
PI 96983	R	R	R	R	R	R	N	S
Essex	S	S	S	S	S	S	S	S
Epps	R	R	R	R	R	R	N	S
V85-5344	R	R	R	R	R	R	N	S

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4) Identification of single genes controlling resistance to powdery mildew in soybean.

Introduction: Powdery mildew of soybeans caused by the fungus Microsphaera diffusa Cke. & Pk., has occurred frequently in many of the soybean growing areas in the United States (Sinclair, 1982). Dunleavy (1980) reported significant yield losses in naturally infected, field-grown soybeans. In 1974, powdery mildew was first observed on plants of breeding lines of soybeans inoculated with peanut mottle virus in Virginia. Reactions of numerous soybean varieties to this disease have been investigated by various researchers (Demski and Phillips, 1974; Dunleavy, 1980; Leath and Carroll, 1982; Lehman, 1947; Mignucci and Lim, 1980).

Grau and Laurence (1975) evaluated a fairly small F_2 population from a resistant x susceptible cross for reaction to M. diffusa and found that the resistance in cultivar 'Chippewa' was inherited as a single, dominant Mendelian trait. Buzzell and Haas (1978) detected a single major gene pair in cultivar 'Blackhawk' and proposed the gene symbols Rmd and rmd for resistance and susceptibility to M. diffusa. In this paper we report inheritance patterns for two additional sources of resistance to powdery mildew of soybeans.

Material and methods: Four parental cultivars and 204 F_2-derived lines from crosses among these cultivars were scored for reaction to powdery mildew in a soybean mosaic virus nursery infected naturally by a severe epiphytotic of the mildew disease. The mildew-resistant cultivars were 'York' and 'Marshall'. The susceptible cultivars were 'Ogden' and 'Kwanggyo'. F_3 lines were grown in three-foot rows and the parents were included every 50 rows throughout the nursery. Disease readings were taken after the fungus had become established. Each row was visually classified as homozygous resistant (R), segregating (H) (ratios not determined but resistance appeared dominant), or homozygous susceptible (S). The observed frequencies of each class were tested for goodness of fit to the 1:2:1 ratio expected for a single dominant gene for resistance.

Results and discussion: The results from the three crosses are presented in Table 1. The F_3 data from each cross provide an acceptable fit to the 1 R : 2 H : 1 S F_2 genotypic ratio. The pooled data for the three crosses show good agreement and homogeneity. Thus, we tentatively conclude that York and Marshall carry single dominant genes for resistance to powdery mildew.

It is not known whether the genetic control of the resistant reaction of York and Marshall is due to different alleles at the same locus or whether two different loci are involved. Nor are their relationships to the genes in Blackhawk and Chippewa known.

York has been identified previously as a resistant cultivar (Leath et al., 1982) and Ogden as susceptible (Lehman, 1947). Our results are in agreement with those evaluations. There is no previous report on the reaction of cultivars Marshall and Kwanggyo to powdery mildew. In our nursery, Marshall was found to be free of foliar symptoms, but Kwanggyo was heavily infected by the mildew disease. Our preliminary results demonstrate that the single genes carried in York and Marshall could be used as sources of resistance.

There might be an association of disease incidences of powdery mildew and viruses. We observed profuse development of powdery mildew (natural occurrence) in our soybean mosaic virus nursery while the disease was not detected in nearby experimental field plots where the virus was absent. Similarly, an earlier outbreak of powdery mildew was found in a peanut mottle virus nursery, but not in commercial soybean fields (Roane and Roane, 1976). We will continue to assess the relationship between the reactions of soybeans to powdery mildew and viral diseases.

Table 1. Segregation and X^2 tests for reaction of F_3 lines to powdery mildew of soybeans.

Crosses	Class & frequency			X^2	df	P
	R	seg.	S			
York x Kwanggyo (RxS)	14	41	25	3.08	2	.10-.25
Kwanggyo x Marshall (SxR)	19	29	16	0.84	2	.50-.75
Ogden x Marshall (SxR)	21	28	11	3.60	2	.10-.25
Total				7.52	6	
Pooled	54	98	52	0.35	2	.75-.90
Heterogeneity				7.17	4	.10-.25

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1) Studies of three chimeric soybean plants from Glycine max x G. soja crosses.

The occurrence of plant chimeras in nature is a common phenomenon. Chimeras are a direct result of the segregation of normal and mutant organelles into genotypically and phenotypically distinct vegetative sectors. The Soybean Genetic Type Collection lists several mutants, both nuclearly and cytoplasmically inherited, that were identified among progeny of chimeric plants. Progeny of chimeric plants manifest a direct relationship between sector phenotypes and nuclear or cytoplasmic genotypes. We expect chimeras with large yellow leaf area to have more yellow plants among their progeny than do chimeras with small yellow leaf area.

We report studies of three chimeric soybean plants from Glycine max (L. Merr.) x G. soja (Sieb. and Zucc.) crosses. Table 1 gives the origin of these three chimeric plants.

Table 1. History of three chimeric soybean plants from Glycine max x G. soja crosses

Mutant No.	Description
1	Chimera found in plot 3128, G. soja BC experiment, Ames 1985 harvest, plant number AX2950-1-2-2; AX2950 = LN78-1136 x PI 407298, LN78-1136 (=Hack) = L70T-543G x K1028.
2.	Chimera found in plot 3501, G. soja BC experiment, Ames 1985 harvest, plant number AX2951-2-1-3; AX2951 = Pride B216 x PI 424004A.
3.	Chimera found in plot 4019, G. soja BC experiment, Ames 1985 harvest, plant number AX2952-29-2-3; AX2952 = A81-356025 x PI 468916, A81-356025 = A76-202015 x C1545.

The general procedure in the backcrossing was:

Ames 1983	F_1 seed obtained, used G. soja flowers as male parent.
Puerto Rico Jan. 1984	F_2 seed and BC_1F_1 seed obtained; G. max was used as male parent for BC.

Ames 1984	25 BC_2F_1 seed obtained and BC_2F_1 seed harvested separately from each BC_1F_1 plant. BC_1F_2 seed harvested.
Puerto Rico Nov. 1984	Obtained BC_3F_1 seed and BC_2F_2 seed. Harvested BC_3F_1 seed from each BC_2F_1 plant separately.
Puerto Rico Feb. 1985	BC_3F_1 seed planted, BC_3F_2 seed harvested from individual BC_3F_1 plants.
Ames 1985	BC_3F_2 seed from BC_3F_1 plants planted in single 5 ft. progeny rows at 4 seed/ft.

As indicated in Table 1, a single chimeric plant was observed in three different progeny rows. The results from self-pollination of these plants are presented in Table 2.

Table 2. Progeny of three chimeric soybean plants from Glycine max x G. soja crosses

Plot no.	Mutant no.	Number of plants and phenotype
A86-400	1	3 chimeras 816 green 139 yellow (viable)
A86-401	2	no chimeras 630 green 108 yellow (lethal)
A86-402	3	1 chimera 787 green 173 yellow (lethal)

A86-400: A total of 51 green plants from A86-400 were single-plant threshed. Forty-four plants were true breeding and gave 16,019 green plants. Seven plants gave 1,611 green plants and 21 yellow plants among progeny. We don't know if these seven plants were lightly chimeric and the green:yellow ratio represents chimeras that had very little yellow leaf area or whether there is a reduction in transmission of the chromosome with the 'yellow factor'. Remnant seed from three of these seven plants are available.

The yellow plants in plot number A86-400 were used as male and female parents in crosses with 'Williams'. All crosses with yellow plants as female parent aborted, probably because the yellow plants were very weak and produced few self-pollinated seed. Ten hybrid seed with Williams as female parent were obtained.

The F_1 seed of Williams x A86-400 yellow were planted as A87-50. Progeny of 10 F_1 plants gave all green F_2 plants but segregation of purple and white

flower color indicated successful hybridization. Remnant F_2 seed will be grown in 1988 and F_2 plants will be single-plant threshed. The F_3 progeny will be evaluated in the sandbench.

Seed from yellow plants of A86-400 were planted in the greenhouse as A87-485. Yellow plants were used as female parent in crosses with Williams and several other white-flower lines. All F_1 plants were yellow (A88g-52). The F_2 seed will be planted and F_2 plant color and flower color will be classified.

Our observations indicate that the yellow plants observed in A86-400 have cytoplasmic inheritance. Additional data are needed to confirm our hypothesis.

A86-401: The yellow plants in plot number A86-401 were dead about 14 days after emergence.

A total of 51 green plants from A86-401 were single-plant threshed. All plants were true breeding and gave 10,532 green plants.

Because no yellow plants survived and no chimeras were found, this yellow mutant has been lost.

A86-402: The yellow plants in plot number A86-402 were dead about 7 days after emergence.

A total of 49 green plants from A86-402 were single-plant threshed. All plants were true breeding and gave 8846 green plants. Progeny of the one chimera will be planted in the sandbench and the chimeras and yellows saved. It might be possible to graft the yellows onto actively growing green plants and rescue them.

Summary: A possible cytoplasmically inherited yellow mutant has been identified in A86-400. The yellow plants in A86-401 and A86-402 were seedling lethals. Progeny of green plants in A86-401 and A86-402 gave all green plants, precluding genetic studies. We could not determine whether the yellow of A86-401 and A86-402 were dominant lethals or cytoplasmically inherited lethals.

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2) Reversion frequency of a mutable allele of the w4 locus in soybean is independent of temperature.

The Asgrow Mutable line of soybean carries an unstable recessive ('mutable') allele of the w4 locus that conditions anthocyanin pigmentation (Groose and Palmer, 1987; Groose et al., 1988). The mutable allele reverts at high frequency from a recessive form to a stable dominant form. The instability of the mutable allele suggests the action of a transposable element.

Temperature affects the behavior of many mutable alleles in plants (Fincham and Sastry, 1974). Cool temperatures often enhance reversion frequencies. In soybean, temperature affects the timing and frequency of mutation of the Y18-m allele of the y18 locus for chlorophyll pigmentation (Sheridan and Palmer, 1977). Like the unstable allele of the w4 locus, the Y18-m allele is thought to be controlled by a transposable element (Peterson and Weber, 1969). The objective of the present study was to determine whether reversion of the mutable allele of the w4 locus is affected by temperature.

Materials and Methods: Three experiments were conducted as follows:

Experiment #1 was designed to determine the effect of temperature on the frequency of reversion of the mutable allele of the w4 locus during the development of standard petals of mutable flowers. Seed were germinated and plants were grown in growth chambers for 8 weeks under continuous light at 30C. Flowering was induced by growing plants for 2 weeks under a day:night regime of 16 hours light at 30C : 8 hours dark at 24C. Each plant was then subjected to one of four different temperature regimes (Table 1) for a period of 10 days. All newly-developed mutable flowers (i.e., that opened from day 4 through day 10) were examined for revertant (purple) sectors. The experiment was terminated at day 10 because plants at the coolest temperature regime produced only cleistogamous flowers after day 10. Frequency of reversion in the standard petals of mutable flowers was evaluated on the basis of the number of mutable flowers with at least one revertant sector greater than 0.5 mm in length. (Smaller sectors were very numerous and could not be accurately counted.) At the highest temperature regime, flowers were produced on 16 mutable plants, four from each of four F_8 sublines of the Asgrow Mutable line. At each of the other temperature regimes, flowers were produced on 8 mutable plants, two from each of the four F_8 sublines.

Experiment #2 was designed to determine the effect of temperature on the frequency of reversion of the mutable allele in hypocotyls of mutable seedlings. Seed were planted at a depth of 2 cm in sand in growth chambers at two temperature regimes (Table 2). Frequency of reversion in hypocotyls was evaluated at 10 days after germination by counting the number of revertant sectors (purple flecks) on a 1 cm section of hypocotyl centered on a point one-fourth of the distance from the surface of the sand to the cotyledonary node. (Evaluation of reversion frequency is more accurately determined near the base of the hypocotyl because anthocyanin pigmentation is more intense in that region and revertant sectors are more distinct.) For each temperature regime, hypocotyls of 81 mutable seedlings (nine from each of nine different F_8 sublines of the Asgrow Mutable line) were evaluated.

Experiment #3 was designed to determine the effect of temperature on the frequency of reversion of the mutable allele in the germ line of mutable flowers. Seed were germinated and plants were grown in growth chambers under continuous light at 30C. Flowering was induced by growing plants for 2 weeks under a day:night regime of 16 hours light at 30C : 8 hours dark at 24C. All open flowers and developing pods were removed and each plant was then subjected to one of two different temperature regimes (Table 3). Newly opened mutable flowers were allowed to set seed. After flowering was completed and seed were set (6 weeks), plants were moved to a greenhouse for seed maturation. Frequency of reversion in the germ line of mutable flowers

was evaluated on the basis of the frequency of germinally revertant progeny. (Germinal revertants have wild-type purple hypocotyls; nonrevertants have mutable hypocotyls.) At each temperature regime, flowers were produced on 8 mutable plants, two from each of four F_8 sublines of the the Asgrow Mutable line.

Results:

Experiment #1: Reversion frequencies in standard petals at the four temperature regimes are presented in Table 1. A chi-square test of independence indicated no significant differences among the four treatments ($X^2 = 5.083$, 3 df, $0.10 < P < 0.25$).

Table 1. Frequency of reversion in standard petals of mutable flowers produced on plants grown at four different temperature regimes

Temperature regime		No.	No. petals		
Day (16 hr)	Night (8 hrs)	plants	Total	Sectored	Reversion frequency (%)
30C	24C	16	1252	79	6.31
27C	15C	8	243	23	9.47
23C	15C	8	698	54	7.74
20C	15C	8	365	20	5.48

Experiment #2: A higher mean number of revertant sectors per 1-cm segment of hypocotyl was observed for seedlings germinated at the lower temperature regime (Table 2). However, this may be attributed to the fact that hypocotyls of these seedlings were shorter (Table 2). These hypocotyls appeared to have the same number of cells as the hypocotyls of seedlings germinated at the higher temperature regime, but the cells were less elongate. Thus, a greater number of cells per unit length could account for the higher number of revertant sectors observed in the 1cm segments from seedlings germinated at the lower temperature regime.

Table 2. Reversion in hypocotyls of mutable seedlings germinated at two different temperature regimes

Temperature regime		Mean number of revertant sectors per 1cm segment of hypocotyl	Mean hypocotyl length (mm)
Day (16 hr)	Night (8 hr)		
30C	24C	50.14	32.84
20C	15C	77.72	23.90

Experiment #3: Reversion frequencies in the germ line of mutable flowers at the two temperature regimes are presented in Table 3. A chi-square test of independence indicated no significant difference between the two treatments (X^2 = 1.075,1 df, 0.25 < P < 0.50).

Table 3. Frequency of reversion in the germ line of mutable flowers produced on plants grown at two different temperature regimes

Temperature regime		No. of progeny		
Day (16 hr)	Night (8 hr)	Revertant	Mutable	Reversion frequency (%)
30C	24C	43	465	8.50
20C	15C	68	595	10.26

Discussion: The reversion frequencies measured in the standard petals of mutable flowers (Experiment #1), hypocotyls of mutable seedlings (Experiment #2), and in the germ line of mutable flowers (Experiment #3) represent reversion relatively late in the development of these tissues. The results of these experiments indicate that reversion of the mutable allele of the w4 locus in these tissues in the later stages of development is independent of temperature.

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3) Construction of a random recombinant DNA library that is primarily single copy sequences

Introduction: Eukaryotic genomes are a mixture of a single-copy and repeated sequences. Since many genes are present only once per genome, separating single-copy from repeated DNA results in an enrichment of gene coding sequences. When establishing a restriction fragment length polymorphism (RFLP) genetic map, single-copy DNA clones are required. Different approaches have been used to identify these and to avoid CNA clones containing repetitive sequences. Construction of cDNA libraries is a strategy that uses mRNA to directly clone gene-coding sequences and, thereby, unique DNA. Random DNA libraries can also be used if the clones are screened for copy number by hybridization to total genomic DNA. Typically, uniform amounts of cloned DNAs are hybridized to radioactive genomic DNA. Since repetitive sequences are present more frequently in the radioactive genomic DNA than are single-copy sequences, clones homologous to repeated sequences anneal to larger amounts of the radioactive DNA. This can be detected by autoradiography and the single-copy clones selected by their lack of hybridization signal. Both of these techniques have drawbacks. The cloning of cDNA is a complex procedure that does not result in a random population of DNAs (e.g., one cDNA may be present at a high frequency.) Typically, cDNA clones nave small DNA inserts that make poor probes. Random DNA libraries are tedious to screen for copy number and the screening process often fails to distinguish between single and low (ca. 20 copies per genome) copy sequences.

Plant DNA is highly modified by methylation of the cytosine base. When the sequence CpG or CpXpG is present (p = phosphodiester bond, X = any nucleotide) plant enzymes methylate the cytosine with a high frequency. It has been noted that DNA sequences that contain genes are modified to a lesser extent. Many restriction enzymes will not digest DNA that has methylated cytosines. We have used a methylation-sensitive restriction enzyme (PstI), which discriminates between modified and unmodified DNA, to construct a library of recombinant DNA that is primarily single-copy DNA sequences. We report here the details of that construction and the purity of the library.

Methods: Total DNA was isolated from the leaves of soybean seedlings as described elsewhere in this volume. This DNA was digested with a 20-fold excess of the restriction enzyme PstI (recognition sequence = CTGCAG) for 1 hour. Restricted DNA fragments were separated in low meltagarose (BRL #5517) by electrophoresis. Fragments were isolated by phenol extraction. A plasmid vector, pBS+ (3770 Tansy Street, San Diego, CA) was digested with PstI, phenol extracted to inactivate the PstI, and then ligated with the genomic PstI DNA fragments. T4 DNA ligase from BRL was used with the buffer provided. Recombinant molecules where transformed in the E. coli strain DH5 alpha by the technique of Alexander et al. (1984) The bacteria were spread on agar broth plates containing ampicillin and X-gal. Ampicillin-resistant colonies that contained a recombinant plasmid were white, while others were blue. White colonies were picked onto a new agar plate for purification. Plasmid was isolated from each colony and characterized by PstI digestion and agarose gel electrophoresis. The copy number of each plasmid was estimated by Southern blotting of the PstI-digested DNA and then probing with radioactive genomic DNA.

Results and discussion: We have generated a plasmid library that contains >10,000 DNA clones. Characterization of these clones revealed a variety of insert sizes between 0.5 and 3.0 Kb When 29 isolates were probed with total genomic DNA, only 4 hybridized to any extent. Three contained 1.9 Kb PstI fragments; the other, 1.2 Kb. The size of one fragment (1.9 Kb) corresponds to a known chloroplast PstI fragment. Since chloroplast DNA is hypomethylated, it wouldn't be surprising that chloroplast DNA would be present. Ribosomal DNA is another repeated class that is not heavily methylated. The 1.2 Kb fragment could be a rDNA clone. Both types were discarded. We have used the remaining clones as probes and found that the hybridization patterns were simple (only a few bands). A simple hybridization pattern is consistent with a single-copy probe. Better evidence comes from RFLP studies on F2 populations, where these bands segregate like simple Mendelian markers (Dr. K. G. Lark, pers. comm.). Therefore, single-copy clones comprise about 85% of the recombinant plasmids. Libraries enriched for single-copy DNA are valuable aids in generating RFLP genetic maps. In the future such libraries could also be used for cloning genes. Screening an enriched library would be faster than screening a random library since the frequency of coding sequences would be higher.

We would like to acknowledge Dr. Benjamin Burr who first used this strategy with maize, and Dr. David Grant who suggested its use in soybean.

Reference

Alexander, D. C., T. D. McKnight and B. G. Williams. 1984. A simplified and efficient vector-primer cDNA cloning system. Gene 31:79-89.

Paul Keim
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4) Use of DNA clones from different species to detect homologous soybean sequences.

Introduction: The W4 locus of soybean is involved in the synthesis of anthocyanins. A mutable allele has been described that appears to have resulted from a transposable element insertion (Groose and Palmer, 1986). The biochemical pathway for anthocyanin biosynthesis has been studied extensively in several plants and some of the genes have been molecularly cloned. We have used such "heterologous" clones to probe soybean DNA in an attempt to isolate the soybean W4 gene. If the mutable allele contains an insertion, a restriction fragment length polymorphism (RFLP) would be detected between mutable and revertant sister lines. We report here preliminary results using different hybridization conditions and five different anthocyanin gene clones.

Methods: DNA was isolated from the leaves of mutable and revertant sister lines and digested with four different restriction enzymes. DNA fragments were separated according to size by agarose gel electrophoresis and transferred to a nylon membrane. "Heterologous" probes were radioactively

labelled using a random primer kit (Boehringer-Mannheim No. 1004 760). Conditions for the annealing of radioactive probes were designed to allow hybridization of partially homologous probes.

Membranes were prehybridized and hybridized at 43° C in 45% formamide, 5X SSC, 50 mM phosphate buffer pH=6.7, 10% dextran sulfate, 5X Denhart's, 0.5% SDS. No salmon sperm DNA was added to this mixture. These conditions will allow sequences of >80% homology to hybridize (see Davis et al., 1980). Membranes were washed under two stringency conditions by altering the temperature and salt concentrations: low stringency washes were at 43° C in 2X SSC with 0.5% SDS; high stringency washes were at 60° C in 0.1 X SSC with 0.5% SDS. Low stringency washes will allow sequences of >65% homology to remain annealed, but high stringency washes require >91% sequence homology.

DNA clones were kindly provided by a number of researchers: Dr. C. Lamb provided the chalcone isomerase cDNA clone from Phaseolus vulgaris, Dr. H. Dooner provided the bz clone from maize, Dr. V. Walbot provided the bz2 clone from maize, Dr. C. Martin provided the pallida clone from Antirrhinum, and Dr. I. Somssich provided the chalcone synthase gene from parsley.

Results and discussion: Hybridization signals from chalcone synthase and chalcone isomerase were easily detected under high stringency conditions. Chalcone synthase appears to be a multigene family in soybean. Depending upon the particular restriction enzyme used, four to eight bands were detected in these DNAs. In none of the four digestions was there a polymorphism between the revertant and mutable lines. In addition, it seems unlikely that a single insertion would result in total blockage of anthocyanin production when a multigene family is involved. We conclude that chalcone synthase is not the W4 locus. Chalcone isomerase gave a very simple pattern of hybridization that involved no more than two bands with any enzyme. No restriction fragment polymorphisms were detected with chalcone isomerase suggesting that this is not the W4 gene either.

The remaining probes detected only faint homology under low stringency conditions and detected no polymorphisms between mutable and revertant sister lines. The faint hybridization detected under these conditions may not be the result of probe annealing to the bonafide soybean gene, but due to non-specific hybridization. Because of this, we cannot rule out these genes as the W4 locus. While these clones do not seem to be useful for detecting homologous sequences by Southern hybridization, they could be used to detect homologous sequences in recombinant DNA libraries. The amount of single-copy DNA present on a Southern blot of total DNA digests is much less than would be present when probing a library.

The homology of genes between different organisms is a function of both the taxonomic distance and the functional constraints of the individual genes upon the sequence. The lack of hybridization by the maize clones may be due to taxonomic distance, since maize is in a different class (Monocotyledoneae) than soybeans (Dicotyledoneae). While Antirrhinum is in the Dicotyledoneae, it is in a separate subclass (Tubiflorae) from soybean (subclass Polypetalae). This may explain the lack of homology to the pallida clone. Parsley, on the other hand, is a member of the Polypetalae (same as soybean) and the chalcone

isomerase probe detected homologous sequences in soybean DNA. Phaseolus is closely related to soybean, being a member of the same tribe of the Leguminosae and consequently the chalcone isomerase probe yields a strong hybridization signal. With these five clones, our ability to use them exactly paralleled the taxonomic distance between the species. Taxonomically, DNA clones from within the same subclass hybridized well with soybean DNA.

References

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Groose, R. W. and R. G. Palmer. 1986. Phenotype of an unstable mutation for anthocyanin pigmentation in soybean. Agron. Abstr. 1986:15.

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5) A rapid protocol for isolating soybean DNA.

In the study of restriction fragment length polymorphisms, DNA must be isolated from a large number of individual plants or lines. Procedures must be rapid and cheap but yield large quantities of DNA that is digestible by restriction enzymes without degradation. Cesium chloride centrifugation is commonly used for purifying DNA, but when more than a few samples are involved, it is tedious and expensive. We present here a protocol that avoids cesium chloride and takes about a day to isolate milligram quantities of DNA.

Freeze-dried leaves are used to isolated DNA. When harvesting leaves, try to pick young expanding leaves. More mature leaves will work, but yield less DNA. Leaves can be harvested at a field site and then transported to the laboratory on wet ice. Freeze leaves in liquid nitrogen, making sure that they're frozen solid. Lyophilyze them for 1-2 days. For long-term storage, we keep the leaves at -20C.

Because field-grown plants have more secondary compounds and larger amounts of starch, DNA isolation is best done from greenhouse-grown plants. Lines with equivalent genotypes (i.e., inbred lines) can be grown in sand and young leaves harvested from scores of seedlings. When single-plant DNA is required, harvesting leaves periodically works well. Field-grown material will yield good DNA, but a few more purification steps are required (see below). The following protocol is adapted from several protocols developed in other laboratories (Saghai-Maroof et al., 1984).

DNA isolation protocol:

1. Freeze-dried leaves are placed into a sample mill (Tekmar A-10) until full (1-2 g). Grind until leaves are a very fine powder. The time required varies from sample to sample but is generally 15 to 30 sec.

2. One gram of milled tissue is placed in a 50 mL tube (we use USA Plastics #1554BS). Add 25 mL of extraction buffer (EB) and mix until the tissue is suspended.

   EB: 50 mM Tris pH=8.0
   1.0 % CTAB (Hexadecyltrimethylammonium Bromide)
   50 mM EDTA (Ethylenediaminetetraacetic Acid)
   1 mM 1,10 o-Phenanthroline
   0.7 M NaCl
   0.1 % beta-Mercaptoethanol

3. Set this slurry in a 60°C water bath for 1 hr. Once or twice during the hour gently mix the slurry by inverting the tubes. Remove the samples from the water bath and allow to cool for several minutes at room temperature.

4. Add 20 mL of chloroform/isoamyl alcohol (24/1) and mix by inverting the tubes until the phases are thoroughly mixed.

5. Separate the phases by centrifugation in a swinging bucket rotor for 10 min at >3,500 g. Remove the upper phase (aqueous) using a disposable pipette (with the tip broken off to form a large orifice) and a pipetting bulb. Frequently the insoluble material between phases is firm enough to allow the aqueous phase to be decanted away from the organic phase. Place the aqueous phase in a new tube and add 2/3 volume of isopropanol. Mix by 2 or 3 inversions.

6. A clot of DNA will form that is transferred (with a glass rod) to a new tube containing 20 mL of 80% ethanol + 15 mM ammonium acetate (pH=7.5). After >20 min transfer the DNA to a 1.5 mL microfuge tube and spin briefly. Remove any residual ethanol with a capillary tube and then dry in vacu or on the bench top. Next add 0.75 mL of TE (10 mM Tris pH=8, 1 mM EDTA) and 20 micrograms of RNase A (heat-treated to destroy DNases). Allow the DNA to resuspend overnight at room temperature. For field-grown plants continue to step 8.

7. Depending upon the starting material, the yield will range from 200 micrograms to several milligrams. We determine the sample absorbance at 260 nanometers to roughly quantify the DNA (1 O.D. equals 50 micrograms per milliliter) and then dilute the sample to a concentration of 500 micrograms per milliliter. This DNA is ready for digestion with restriction enzymes.

Field-grown Plants

8. Take RNase-treated DNA and dilute to 20 mL with TE. Add 3.3 mL of 5 M NaCl and mix. Next add 2.5 mL of 10% CTAB, 50 mM Tris, 0.7 M NaCl and mix. Extract with 17.5 mL of chloroform (as in step 4). Separate the phases by centrifugation (as before). Move the upper aqueous phase to a clean tube.

9. Add an equal volume of precipitation buffer (50 mM Tris pH=8, 1% CTAB) to the DNA solution and mix. Set this at room temperature overnight to allow the precipitate to form. Pellet the precipitated DNA by centrifugation at 3,500 g for 10 min. Pour off the supernatant and dry the pellet on the bench top.

10. Resuspend the pellet by adding 2 mL of 5 M NaCl and gently mixing. After an hour add 8 mL of TE. CTAB pellets can only be resuspended in high salt. Higher temperatures (60°C) and gentle pipetting may aid the process. Material that resists resuspension can be removed by centrifugation and discarded.

11. Precipitate the DNA with 25 mL of 95% ethanol. Transfer the DNA clot (with a glass rod) to a new tube with 80% ethanol and 15 mM ammonium acetate. After 20 min transfer the clot to a microfuge tube and dry the DNA (as in step 6). Resuspend the DNA clot in TE, quantify, and dilute to the appropriate volume (as in step 7).

Reference

Saghai-Maroof, M.A., K.M. Soliman, R. A. Jorgensen and R. W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. USA vol 81:8014-8019.

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6) 245 A sterile mutant found in a genetically unstable line of soybean.

In a field grow-out of the Asgrow Mutable line of soybean (Groose and Palmer, 1986) four green plants were found at harvest. Two of the plants bore a single one-seeded pod each, while the other two plants were completely sterile. One of the seeds gave rise to a 44-chromosome plant, which died before the second trifolioliate expanded. The other seed produced a 40-chromosome plant, which set 14 seeds. All 14 seeds produced mature plants in the field the summer of 1987. Pollen from each of these plants was stained with I_2KI and viewed under 125x magnification. The cytoplasm from pollen of seven of the plants was dark and severely collapsed inside of the cell wall. The remaining seven plants produced fully normal pollen and had good seed set. All of the plants with abnormal pollen remained green until harvest, and all were barren except for two plants with one one-seeded pod each. Only one of these seed produced a plant. The root-tip of this weak plant had 44 chromosomes, and at the time of this writing was at the second-trifolioliate stage in the greenhouse. Any progeny of this plant, as well as progeny of the seven normal plants, will be evaluated the summer of 1988.

Reference

Groose, R. W. and R. G. Palmer. 1986. Phenotype of an unstable mutation for anthocyanin pigmentation in soybean. Agron. Abstr. 1986:15.

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R. G. Palmer - USDA

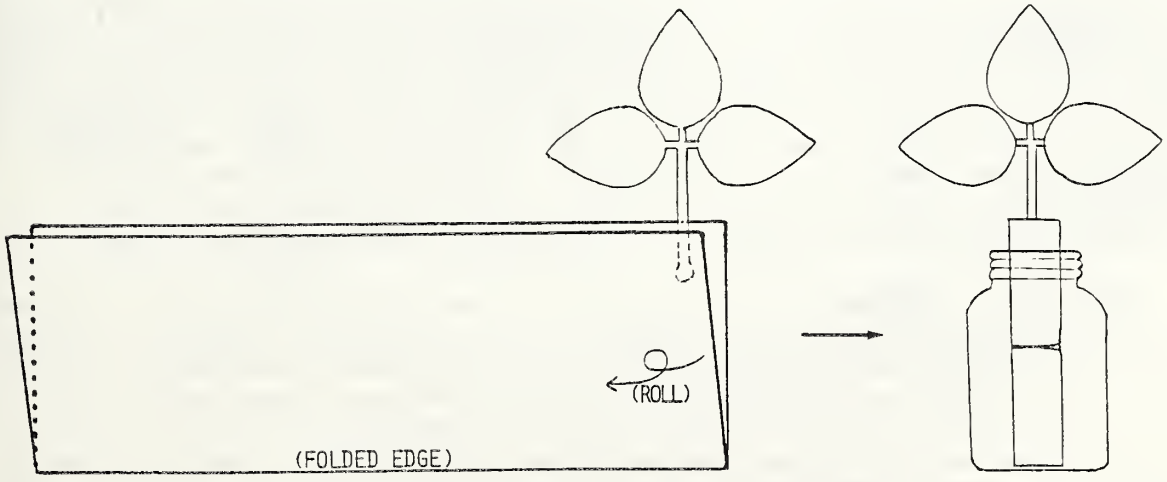
27)

7) A petiole-rooting technique for soybean chromosome observations//

Through replicated testing we have developed a protocol for the rapid production of roots on petiole cuttings. This technique will be useful when tips are needed for chromosome analysis from a seedling that has been planted in or transplanted to soil, rather than being on germination paper as suggested by Palmer and Heer (1973). This method produces strong, clean roots with good mitotic indices.

The leaf-with-petiole cuttings should include a portion of the node in order to provide meristematic tissue for root initiation. Care should be taken not to damage the donor plant irreparably. The cutting should immediately be placed in water or a solution of 0.004% Captan (one tablespoon in one gallon) to prevent drying while awaiting transfer to the rooting medium. The Captan serves to inhibit decay of the petiole during the rooting period.

The same nontoxic germination paper that worked well for seed germination (Burris and Fehr, 1971; Palmer and Heer, 1973) worked well for root production on petioles. One pre-moistened 10" by 15" (25.4 x 38.1 cm) sheet should be used for each leaf-with-petiole, folded in half along the longer mid-line. The enlarged portion of the petiole tip should be dipped in 0.3% (number 30) IAA powder to speed root production. (Lower concentrations were found to be less effective, while higher concentrations produce an abundance of weaker roots.) After tapping off excess hormone powder, the petiole should be placed between the two folded halves of toweling, with its tip approximately one inch (2.5 cm) in from each corner edge, leaflets protruding (see diagram). The folded paper should be rolled firmly, starting from the petiole. The finished roll, petiole in the center, can be secured with a snugly fit rubber band. We found that rolling the toweling too tightly produced unhealthy roots, while a loose roll did not produce the thick, strong roots desirable for cytology. A small bottle (3.15 x 7.5 cm) filled with water works well to support the roll and provide moisture.



Since moisture is such an important element to successful petiole rootings, the labeled vials should be placed in a well sealed glass or clear plastic box, with a layer of water in the bottom, for the duration of the germination period. Trials showed that even slight ventilation can result in the death of cuttings. Boxes should be placed in a lighted growth chamber at 20° C. White callus will form on the tips of healthy cuttings in a few days. Ample roots should appear in 7 to 10 days, by which time the root tips should be far enough from the petiole tip to escape any effects the hormone may have on dividing chromosomes.

References

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Palmer, R. G. and Hollys Heer. 1973. A root tip squash technique for soybean chromosomes. Crop Sci. 13:389-391.

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8) The seventh independent mutation at the msl locus.

Genetic studies of male-sterile, female-fertile mutations conducted by Palmer et al. (1978), Yee and Jian (1983). Skorupska and Palmer (1986, 1987) reported six independent mutations at the msl locus.

Origin: Sterile plants were found in a field of the cultivar 'Beeson' in Danbury, Iowa by Marc Albertsen. These plants set a few pods. Progeny of sterile plants segregated for flower color and pubescence color. Observations indicated that seed set on sterile Beeson plants resulted from outcrosses and the male parent is unknown. This would indicate that the original field of Beeson was not a pure cultivar, or that cross pollination occurred with a different variety planted in a nearby field.

Methods: Cytological observation of anthers (stained with I_2KI) showed that sterile plants produced coenocytic pollen grains. Male-sterile, female-fertile mutation msl msl inhibits cytokinesis in meiosis after telophase II, which results in large dark-staining coenocytic microspores. Because of similar microspore abnormalities, we test-crossed the unknown mutant with msl msl (T266). Reciprocal crosses were conducted by using male-sterile, female-fertile plants of msl msl (T266) and ms ms (Danbury) as female parents and known heterozygotes of two mutants as male parents.

Results: Segregation for sterility in the F_1 generation from reciprocal crosses gave a good fit to the expected 1:1 ratio ($P>0.5$) (Table 1). Five F_2 progenies from cross msl msl (T266) x Ms ms (Danbury) and seven F_2 progenies from cross ms ms (Danbury) x Msl msl T266H were observed for sterility segregation. In both F_2 populations from reciprocal crosses the ratio of fertile-to-sterile plants did not differ significantly from a 3:1 ratio (Table 1). Results obtained in F_1 and F_2 generations indicated that mutation ms ms (Danbury) is allelic to the msl locus.

Pod set on sterile ms ms (Danbury) plants was lower than on msl msl (T266) plants. Probably mutant Danbury is not as female fertile as msl msl (T266). The mutant produced 9.2 seed per plant ($\bar{X}$ from two years), where msl msl (T266) gave 13.8 seed per plant (Table 2). We propose to name the new mutation found in cultivar Beeson as msl (Danbury). This mutation is maintained in the Soybean Genetic Type Collection as T290.

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Table 1. Segregation in the F_1 and F_2 generations from crosses at the msl locus

Cross Combination	Generation	Observed Fertile	Observed Sterile	Observed Total	Expected Fertile	Expected Sterile	X^2	P
msl msl (T266) x Ms ms(Danbury)								
	F_1	5	4	9	4.5	4.5	0.11	>0.50
	F_2	515	152	667	500.25	166.75	1.74	>0.10
Total	(5 df)						3.05	>0.50
Homogeneity	(4 df)						1.31	>0.75
ms ms(Danbury) x Msl msl(T266HA)								
	F_1	7	5	12	6	6	0.33	>0.50
	F_2	480	165	645	483.75	161.25	0.11	>0.50
Total	(7 df)						0.82	>0.99
Homogeneity	(6 df)						0.71	>0.99

Table 2. Seed set on sterile plants of ms ms(Danbury), and msl msl (T266)

Mutant	Year	Number of sterile plants	Average number Seed/plant	Average number Seed/pod
msl msl(T266)	1986	50	11.6	2.0
	1987	30	17.6	1.6
			13.8	1.8
ms ms (Danbury)	1986	25	9.2	1.6
	1987	25	9.2	1.6
			9.2	1.6

References

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Skorupska, H. and R. G. Palmer. 1987. New independent mutations: ms1 (Ames 2). Soybean Genet. Newsl. 14:181-183.

Yee, C. C. and L. Jian. 1983. Allelism tests of Shennong male-sterile soybean L-78-387. Second Assembly Symp. Genet. Soc. China. Sec. 4. No. 053.

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9) A third independent mutation at the ms3 locus.

A new male-sterile, female-fertile mutant was used for allelism test to determine whether this mutant arose at a new locus or represents an independent mutation at a previously described locus.

Origin: A male-sterile female-fertile mutation was found by William Davis in Plainview, TX in the F_2 generation of the cross ('Viking' x 1 Classic II') x ('Mitchell" x 'Columbus'). "Viking" was a private line (Merit x Amsoy), not the Illinois "Viking" of present-day use. For our records this mutant was called BDI.

Methods: Test crosses were conducted, with recessive homozygotes of the new mutation used as a female parent and known heterozygotes at the ms2, ms3, ms4 loci used as male parents. Reciprocal crosses were done for the ms2 locus. Segregations for sterility in F_1 and F_2 generations were observed. (In F_1, pollen staining in I_2KI; in F_2, classification of plants at maturity).

Results: From test crosses of the BDI mutant with Ms2 ms2 and Ms4 ms4 genotypes, only fertile plants were observed in the F_1 generation. Eight fertile and seven sterile plants were identified in the F_1 from the cross with Ms3 ms3 mutation. These data gave a good fit to 1:1 ratio, and pointed out that the new mutation is allelic to the ms3 locus (Table 1). Further observation in the F_2 generation confirmed this hypothesis. The F_2 progenies from crosses with Ms2 ms2 and Ms4 ms4 segregated for sterility in the ratio 3:1 and 9:7 (Table 2, 3, 4, 6). None of eight F_2 progenies from crosses with Ms3 ms3 segregated in a 9:7 pattern. Among 860 plants, 651 were fertile and 209 were sterile, fitting a 3:1 ratio (X^2-0.22, $P > 0.50$) (Table 5).

Two independent mutations were located previously at ms3 locus. Mutation ms3 (Washington) was found in the F_3 generation of 'Calland' x 'Cutler' cross (Palmer et al., 1980). Mutant ms3 (Flanagan) was found in cv 'Wabash'

(Chaudhari and Davis, 1977; Graybosch and Palmer, 1987). Allelism tests conducted with this new male-sterile, female-fertile mutant showed that this mutation is at the ms3 locus. We propose to name this mutation as ms3 (Plainview). This mutation is maintained in the Soybean Genetic Type Collection as T291.

Table 1. Segregation for sterility in F_1 generation from test crosses with soybean BDI sterile mutant

Cross combination	Number of F_1 plants			Expected ratio	X^2	P
	Fertile	Sterile	Total			
ms ms BDI x Ms2 ms2	32	0	32	16:16	----	----
ms2 ms2 x Ms ms BDI	20	0	20	10:10	----	----
ms ms BDI x Ms3 ms3	8	7	15	7.5:7.5	0.07	>0.75
ms ms BDI x Ms4 ms4	15	0	15	7.5:7.5	----	----

Table 2. Number of F_2 families segregating for sterility in the ratio 3:1 and 9:7 in soybeans

Cross combination	F_2(3:1)	F_2(9:7)	Total	Expected ratio	X^2	P
ms ms BDI x Ms2 ms2	19	13	32	16:16	1.1	>0.25
ms2 ms2 x Ms ms BDI	11	9	20	10:10	---	----
ms ms BDI x Ms3 ms3	8	0	8	4:4	---	----
ms ms BDI x Ms4 ms4	9	6	15	7.5:7.5	0.6	>0.25

Table 3. Segregation in the F_2 population of the ms2 ms2 x Ms ms BDI cross in soybean

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	966	333	1299	591	526	1117
Expected	974.25	324.75	1299	628.3	488.6	1117
X^2 pooled	(1 df)	0.27	P>0.50	(1 df)	5.16	P>0.01
X^2 total	(11 df)	2.27	P>0.99	(9 df)	9.45	P>0.25
X^2 homogeneity	(10 df)	1.99	P>0.99	(8 df)	4.28	P>0.75

Table 4. Segregation in the F_2 population of the ms ms BDI x Ms_2 ms_2 in soybeans

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	1758	556	2314	947	760	1707
Expected	1735.5	578.5	2314	960.2	746.8	1707
X^2 pooled	(1 df)	1.17	P>0.25	(1 df)	0.41	P>0.50
X^2 total	(19 df)	7.33	P>0.99	(13 df)	3.87	P>0.99
X^2 homogeneity	(18 df)	6.16	P>0.99	(12 df)	3.46	P>0.99

Table 5. Segregation in the F_2 population of ms BDI x Ms3 ms3 cross in soybean

	Fertile	Sterile	Total
Observed	651	209	860
Expected	645	215	860
X^2_2 pooled	(1 df)	0.22	P>0.50
X^2_2 total	(8 df)	0.82	P>0.99
X^2 homogeneity	(7 df)	0.60	P>0.99

Table 6. Segregation in the F_2 population of the ms ms BDI x Ms4 ms4 cross in soybean

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	894	286	1180	473	388	861
Expected	885	295	1180	484.3	376.7	861
X^2_2 pooled	(1 df)	0.36	P>0.50	(1 df)	0.60	P>0.25
X^2_2 total	(9 df)	9.57	P>0.25	(6 df)	2.09	P>0.90
X^2 homogeneity	(8 df)	8.21	P>0.25	(5 df)	1.48	P>0.90

References

Chaudhari, H. K. and W. H. Davis. 1977. A new male-sterile strain in Wabash soybeans. J. Hered. 68:266-267.

Graybosch, R. A. and R. G. Palmer. 1987. Analysis of a male-sterile character in Glycine max (L.) Merr. cv Wabash (soybean). J. Hered. 78:66-70.

Palmer, R. G., C. W. Johns and P. S. Muir. 1980. Genetics and cytology of the ms3 male-sterile soybean. J. Hered. 71:343-348.

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10) Localization of a male-sterile, female-fertile mutation at ms4 locus.

Thus far only one mutation, T274H, has been identified at the ms4 locus. Male sterility caused by the ms4 gene is related to the failure of postmeiotic cytokinesis, which can be either absent or delayed (Delannay and Palmer, 1982). Graybosch and Palmer (1984, 1985) showed that ms4 male-sterile plants were capable of a low rate of seed production in the absence of pollinating vectors.

We observed that one male-sterile, female-fertile mutant formed degenerated, clumped pollen grains along with normally stained microspores. Allelism tests were conducted to determine the inheritance of this mutation.

Origin: Mutant was identified by William Davis in Fisher, AR, in the cultivar 'Corsoy'[1]. In our records this mutant was called BDII.

Method: Test crosses were conducted by using recessive homozygotes at the ms2, ms3 and ms4 loci as female parents and known heterozygotes of the new mutant as male parent. Sterility segregation in F_1 (pollen staining in I_2KI), and in F_2 generations (classification of plants at maturity) was observed.

Results: F_1 plants from crosses of the BDII mutant with ms2 ms2 and ms3 ms3 genotypes were fertile. We observed 27 fertile and 10 sterile plants from crosses to test for allelism at the ms4 locus. X^2 value for 1:1 ratio was 7.81, and probability was lower than expected (Table 1). Segregation for sterility of F_2 families showed that the new mutation is not allelic to the ms2 or ms3 genes. Twenty-two and nine progenies, respectively, from crosses ms2 ms2 and ms3 ms3 with the BDII mutant segregated with a 9:7 ratio (Table 2, 3, 4). Twenty-seven progenies from crosses with ms4 ms4 segregated only in a 3:1 ratio (Table 4). In the F_2 population, 2973 plants were classified as fertile and 945 as sterile. X^2 was 1.62 and probability >0.10. No populations segregated 9:7 (Table 5). Data of segregation for sterility in the F_2 generation showed that the BDII mutation arose at the ms4 locus. We propose to name the mutant BDII as ms4(Fisher). This mutation is maintained in the Soybean Genetic Type Collection as T292.

Table 1. Segregation for sterility in the F_1 generation from test crosses with BDII sterile mutant in soybean

Cross combination	Number of plants			Expected ratio	X^2	P
	Fertile	Sterile	Total			
ms2 ms2 x Ms ms BDII	54	0	54	27:27	---	---
ms3 ms3 x Ms ms BDII	22	0	22	11:11	---	---
ms4 ms4 x Ms ms BDII	27	10	37	18.5:18.5	7.81	>0.005

Table 2. Number of F_2 families segregating for sterility in the ratio 3:1 and 9:7

Cross combination	F_2(3:1)	F_2 (9:7)	Total	Expected ratio	X^2	P
ms2 ms2 x Ms ms BDII	32	22	54	27:27	1.85	>0.10
ms3 ms3 x Ms ms BDII	13	9	22	11:11	0.72	>0.25
ms4 ms4 x Ms ms BDII	27	0	27	13.5:13.5	---	---

Table 3. Segregation in the F_2 population of the ms_2 ms_2 x Ms ms BDII cross

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	3475	1113	4588	1753	1404	3157
Expected	3441	1147	4588	1775.8	1381.2	3157
X^2 pooled	(1 df)	1.34	P>0.10	(1 df)	0.66	P>0.25
X^2 total	(32 df)	35.96	P>0.10	(22 df)	11.99	P>0.95
X^2 homogeneity	(31 df)	34.61	P>0.25	(21 df)	11.32	P>0.95

Table 4. Segregation in the F_2 population of the ms3 ms3 x Ms ms BDII cross

	Ratio					
	3:1			9:7		
	Fertile	Sterile	Total	Fertile	Sterile	Total
Observed	1086	383	1469	658	602	1260
Expected	1101.75	367.25	1469	708.75	551.25	1260
X^2 pooled	(1 df)	0.90	P>0.25	(1 df)	8.31	P>0.005
X^2 total	(13 df)	3.98	P>0.99	(9 df)	8.87	P>0.25
X^2 homogeneity	(12 df)	3.08	P>0.99	(8 df)	0.56	P>0.99

Table 5. Segregation in the F_2 population of ms4 ms4 x Ms ms BDII cross

	Fertile	Sterile	Total
Observed	2973	945	3918
Expected	2938.5	979.5	3918
X_2^2 pooled	(1 df)	1.62	P >0.10
X_2^2 total	(27 df)	11.99	P >0.99
X^2 homogeneity	(26 df)	10.37	P >0.99

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11) Aberrant segregation of a variegated mutant in soybean. //

Newhouse et al. (1983) reported the existence of a nuclearly inherited variegated leaf mutant in soybean. Palmer (1984) located the variegated mutant to Trisomic A on the basis of comparison of the trisomic- to disomic-segregation ratios. Subsequent studies confirmed that the mutation is inherited as a single recessive allele located on the extra chromosome of Trisomic A (Honeycutt et al., manuscript in review). Crosses of the variegated mutant to an isoline of cultivar 'Hark', which is homozygous for two genes of Linkage Group 11, revealed aberrant segregation of the variegated trait (Honeycutt et al., manuscript in review).

Our objective in this research was to determine: 1) the effect, if any, of different genetic backgrounds on the segregation of the variegated mutation, and 2) if other previously untested genes were located in the Trisomic A linkage group using the variegated mutation as the test marker.

Materials and methods: Seven cultivars and plant introductions were used as the female parent in crosses with the variegated mutant. These seven were chosen on the basis of the following: PI 290.310C, lectin null variant that carries a putative inactive transposable element (Rhodes and Vodkin, 1985); AS 779, a mutable line that carries an active transposable element (Groose et al., 1988); and cv. 'Minsoy' and PI 290.136, which, when crossed produce progenies showing restriction fragment length polymorphisms (Apuya et al.,1988); and cultivars 'Evans', 'Swift', and 'Williams 82', which represent common commercial genotypes. In addition, five genotypes were used as the female parent in crosses with the variegated mutant to test for linkage of the variegated trait to the following: semi-appressed pubescence (pa1 pa1 Pa2 Pa2, PI 79593); dense pubescence (Pd2 Pd2, T264); dense pubescence (Pd1 Pd1, L62-801); glabrous (P1 P1, L62-1377); and nonfluorescent root (Fr3 Fr3, PI 424.078).

Cultivars 'Evans', 'Swift', and 'Williams 82' were crossed to the variegated line KN 1-1, and the F_1 plants were grown in the field during the summer of 1987. F_2 seedlings were classified in the greenhouse sandbench as green or variegated. All other test lines were crossed to line A84-1840-77(1), a mostly yellow variegated line established from the original variegated line KN 1-1. The F_1 plants from these crosses were grown in the field during the summer of 1986, and the F_2 seedlings were grown in the greenhouse sandbench and classified as green or variegated. Classifications of pubescence markers for linkage tests were done by using a dissecting microscope. Classification of root fluorescence was done as described by Delannay and Palmer (1982). Isozymes markers Pgi2, Aco4, Ap, Dia1, and Idh2 were screened using starch gel electrophoresis as described by Cardy and Beversdorf (1984). Statistical calculations were done according to Mather (1951).

Results and discussion: The F_2 segregation ratios of all genotypes crossed with the variegated mutant deviate from the expected 3:1 ratio (Table 1). In all crosses, the deviation from the expected 3:1 ratio could be due either to an excess of green seedlings or a deficiency of variegated seedlings. The greatest average deviation, 10.3:1, occurs in a cross in which 95.4% of the F_2 seeds germinated (Table 1); however, if the remainder (4.6%) of the nongerminated seeds were assumed to give rise to variegated seedlings, then the average segregation ratio would be reduced to 6.6:1, still greater than the expected 3:1 ratio. It is clear, therefore, that poor germinability alone does not account for the deviations in segregation ratios.

Table 1. Summary of F_2 segregation data for plant color in crosses with the variegated mutant as the male parent

Cross	No. F_1 families	Total planted	%Germ.	Total green	Total var.	Range of ratio:1	Avg. ratio:1	X^2(3:1)	Homogeneity[a] X^2
PI290.310C x A84-1840-77(1)	7	2485	80.1	1572	432	2.9-5.0	3.6	12.7**	11.7*
AS779 x A84-1840-77(1)	12	3736	78.9	2394	553	3.3-5.7	4.3	61.1**	9.7
PI290.136 x A84-1840-77(1)	12	5011	94.0	4121	589	4.8-10.6	7.0	392.2**	16.2
Minsoy x A84-1840-77(1)	18	4892	81.9	3245	760	3.0-7.5	4.3	77.6**	25.5
PI79593 x A84-1840-77(1)	15	1050	75.3	669	122	4.0-8.4	5.5	38.7**	2.9
T64 x A84-1840-77(1)	3	875	89.6	662	122	4.5-7.8	5.4	39.3**	34.7*
L62-801 x A84-1840-77(1)	20	1000	60.8	502	113	1.6-17.0	4.4	14.4**	14.9
L62-1377 x A84-1840-77(1)	4	237	95.4	206	20	4.5-37.5	10.3	31.4**	4.7
PI424.078 x A84-1840-77(1)	7	853	79.2	588	88	2.9-9.2	6.7	51.8**	4.4
Swift x KN 1-1	10	1768	93.2	1303	344	3.0-5.2	3.8	14.9**	5.8
Evans x KN 1-1	5	1670	95.2	1236	354	3.3-5.0	3.5	6.4*	2.1
Williams 82 x KN 1-1	22	4946	94.2	3617	1041	2.1-7.7	3.5	17.5**	30.8

[a]Degrees of freedom for homogeneity X^2 are n-2, where n=no. of F_1 families.
* Significant at the 5% level of probability. ** Significant at the 1% level of probability.

Table 2. Tests of homogeneity between ten crosses for the segregation of the variegated mutation

	df	X^2
Total	124	822.84
Deviation	1	535.91
Homogeneity	123	286.93**

**Significant at the 1% level of probability.

Table 3. Linkage tests between five genetic markers and the variegated mutation

Female parent	Marker[a] X^2 (3:1)	Variegated[a] X^2 (3:1)	Joint seg.[a] X^2	Total[b] X^2 (9:3:3:1)
L62-801 (Pd1)	15.48**	14.40**	5.81*	35.69**
T264 (Pd2)	0.11	39.25**	2.80	42.16**
L62-1377 (P1)	0.01	31.44**	0.11	31.55**
PI424.078 (Fr3)	2.56	51.76**	0.85	55.17**
PI79593 (Pa2)	3.64	38.69	10.02**	52.35**

[a] One degree of freedom. [b] Three degrees of freedom.
* Significant at the 5% level of probability.
** Significant at the 1% level of probability.

Table 4. Single locus segregation of five isozyme markers in the cross of PI79593 x variegated

Locus	Exp. seg. ratio	df	X^2
Pgi2	3:1	1	0.87
Aco4	1:2:1	2	6.98*
Ap	1:2:1	2	6.05*
Dia1	1:2:1	2	7.07*
Idh2	1:2:1	2	6.03*

* Significant at the 5% level of probability.

Homogeneity tests indicate that, in 10 of the 12 crosses, F_2 populations are homogeneous with respect to segregation of the variegated trait within the cross (Table 1). The 10 "homogeneous" crosses were compared with one another to determine if the different genetic backgrounds produced the same distorted segregation ratio. The homogeneity test (Table 2) indicates that the crosses produced different segregation ratios for the variegated trait. Although the segregation ratio of each cross deviates from a 3:1 ratio, when compared one to another, these aberrant segregation ratios are not uniform indicating that the genetic background influences the segregation of the variegated trait.

The variegated trait was tested for linkage with five markers. All tests for goodness-of-fit to a 9:3:3:1 ratio were significant (Table 3). The total chi-squares (Table 3) were partitioned to establish the sources of variability because skewed single locus segregation ratios were observed for one or both loci involved in each linkage test. The chi-square for joint segregation suggested that two markers, Pa1 and Pd1, are linked to the variegated trait (Table 3). The product method (Immer and Henderson, 1943) was used to calculate recombination values of 0.31 for Pa2 and variegated and 0.40 for Pd1 and variegated; however, these linkage estimates may be biased by the skewed segregation ratios of some of the loci involved.

Remnant F_2 seeds of the cross of PI79593 (Pa 2) by the variegated mutant were used to test single locus segregation of five isozyme markers. In this cross, Aco4, Ap, Dial, Idh2 (Table 4), and the variegated trait (Table 3) deviate from their expected segregation ratios, while Pgi2 (Table 4) and Pa2 (Table 3) segregate as expected. Thus, the segregation of several loci is differentially affected by some, as yet undetermined, genetic anomaly.

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1) The line PI 132.201 has the same amylase phenotype as 'Chestnut', and may be unstable.

The line PI 132.201 had previously been reported as having the same amylase zymogram as found in some 'Altona' seeds homozygous for the sp-1 (am3-n) allele by Gorman (1983) and was listed by Palmer et al. (1985) as an example of a sp-1 type. Seeds homozygous for the sp-1 allele lack a detectible beta-amylase or corresponding seed protein band (Kiang, 1981; Hildebrand and Hymowitz, 1980). However, upon further testing, seeds from PI 132.201 were found to have identical amylase zymograms in both simple and gradient PAGE gels as seeds from the cultivar Chestnut. Chestnut seeds are homozygous for the Sp1-an (Am3-sw) allele, which conditions a similar zymogram type with a low activity, slow mobility beta-amylase isozyme that can be detected as a seed protein band. PI 132.201 was not tested for the presence of a seed protein band.

F2 seeds from both reciprocal crosses between PI 132.201 and Altona segregated with the PI 132.201 zymogram dominant to the Altona. Both Kiang (1981) and Hildebrand and Hymowitz (1980) found the Sp1-an allele dominant to the Sp-1 allele. Thus it is concluded that PI 132.201 and Chestnut are both homozygous for the Sp1-an allele, although they may have unique origins and are not necessarily identical. These are the only two lines in which this allele has been found, while Altona and PI 68.398 are the only known lines with the Sp-1 allele. These 4 lines have no known relationship with one another. PI 132.201 was obtained via the Netherlands, but its actual geographic origin is unknown. Chestnut was probably selected from alien genetic material in the Russian cultivar Habaro seed lot (R. L. Bernard, pers. comm.) Altona probably represents a new amylase mutant occurring in the single F4 plant from which the cultivar originated, as the parent and sib lines have full activity (Hildebrand and Hymowitz, 1981).

Interestingly, one out of 49 PI 132.201 seeds tested from a greenhouse-grown plant displayed the fully active slow mobility zymogram, as did one of the 422 F2 seeds tested from the PI 132.201 x Altona crosses. While outcrossing can't be ruled out, it seems unlikely for out crossing to be occurring in greenhouse-grown plants, particularly since both the unusual seeds had the slow (Sp1-a) band rather than the fast (Sp1-b) band that most of the other plants being grown had. A seed mixup is also unlikely, since PI 132.201 has distinctive small black seeds, while none of the other lines being used at the time had black seeds except Kingston which is a Sp1-b type. The two seeds did not appear to be unusual in any way except for their amylase type. It is possible that the Sp1-an allele contained by PI 132.201 is unstable and reverts back to the Sp1-a allele. Mobile DNA elements can act to block transcription, resulting in no protein product like sp-1, or reduce transcriptional read through, resulting in low activity types like Sp1-an. Mobile elements, by definition, move, causing new often unstable (high backmutation rates due to excision) alleles where they insert. Thus a plausible, but unsubstantiated explanation for both the origin of these variant amylase types and the two observed full activity seeds is the insertion of a mobile element into the amylase locus. This hypothesis needs to be investigated further.

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1) Near-isolines which differ in seedcoat permeability.

The presence of an impermeable seedcoat inhibits or delays the imbibition of water by the seed. The expression of impermeability is influenced by many factors, including post-harvest drying temperature (Potts et al., 1978), water stress during seed formation (Hill et al., 1986b), seed size (Hill et al., 1986a), and the field environment (Baciu-Miclau, 1970; Hartwig and Potts, 1987; Minor and Paschal, 1982). The impermeable trait has been shown to improve viability of seeds exposed to adverse weather conditions after maturity (Hartwig and Potts, 1987; Potts, 1978) stored under simulated tropical conditions (Minor and Paschal, 1982), or weathered artificially in an incubator (Yaklich and Kulik, 1987). Additionally, impermeability has been associated with reduced incidence of seedborne fungi which cause deterioration of seed quality (Yaklich and Kulik, 1987). Most soybean cultivars that are currently in production have highly permeable seedcoats. Potts (1978) showed that a moderate level of impermeability can be reduced to a level that would allow sufficient germination to meet certification standards by harvesting the seeds with a field combine. The incorporation of an appropriate level of impermeability may improve seed quality, especially in tropical and subtropical environments, without requiring an additional scarification treatment.

Most previous studies on the effects of impermeability have utilized relatively unrelated genotypes. Hartwig and Potts (1987) suggest that in such studies other genetic factors affecting seed quality may be confounded with impermeability. An inheritance study by Kilen and Hartwig (1978) indicated that the impermeable trait may be conditioned by as few as three major genes. Repeated sampling of the variability available in segregating rows as generations are advanced would produce isogenic lines that would minimize confounding effects. The isolines developed could then be used to test directly the effect of various levels of seedcoat permeability on seed quality. The objectives of this study were to select for discrete levels of seedcoat permeability, and stabilize them in improved, closely-related, adapted genotypes for future research.

Plants for this study originated as selections from two crosses: Cross 1, ('Kirby' x ('Forrest'(3) x D77-12480) x F81-5590, and Cross 2, (Kirby x ('Forrest(3) x D77-12480) x F81-5588. The impermeable seedcoat trait was donated by F81-5590 and F81-5588, two advanced F_5 lines selected from the same F_4 row in 1980 and of the parentage D65-8232 x D77-12480. The trait was carried in D65-8232 and traces to PI 163453 (Glycine soja Sieb. and Zucc.). D77-12480 is a selection from 'Tracy' x ('Hill' x PI 159925), and is the source of the "long juvenile" trait donated by PI 159925, which delays flowering under short-day conditions (Cregan and Hartwig, 1984). All plants used in this study exhibited the long juvenile response.

From F_4 plants selected in 1984, F_5 progeny rows were grown at Gainesville Florida (FL) in 1985. From each row, five single plants were selected at maturity and threshed mechanically. Samples of 25 seeds were tested for permeability by soaking in petri dishes that contained germination paper and deionized water. Only seeds that had no obvious defects were tested, and the number still impermeable at 1 and 27 h in water was recorded. The two plants from each F_5 row with the largest difference in percentage of impermeable seeds at 1 h were selected for planting in 1986. Twelve-seed hill plots were planted at Clayton, North Carolina (NC) in 1986, and at maturity five single plants were selected from each hill. Single plants were handled as in 1985. The two selections from each F_6 hill with apparent differences in permeability at 1 h were planted in 12-seed hill plots at Hartsville, South Carolina (SC) and FL in 1987. The plots at SC were sampled as previously (5 plants/hill) while the FL plots were harvested in bulk with a stationary plot thresher. No differences were observed between selections from sibling SC F_7 hills, and data from both were averaged for the SC mean. For permeability testing, four 25-seed samples were taken from each FL bulk. All data were arcsine square root transformed before the data analyses were performed.

Table 1. Mean percentage of impermeable soybean seeds at 1 and 27 h in water for three pairs of near-isolines grown in three environments

		1986 NC		1987 SC		1987 FL		MEAN	
Cross	Line	1 h	27 h	1 h	27 h	1 h	27 h	1 h	27 h
					%				
1	157-13	2	0	10	0	45	40	19	14
	158-15	0	0	0	0	3	2	1	1
2	163-49	0	0	0	0	1	0	0	0
	164-52	49	14	98	82	81	76	76	57
1	177-53	58	19	95	77	80	69	78	55
	178-56	0	0	6	0	9	3	5	1
	LSD .05	16	9	4	5	9	11	--	--

Data from three environments, which included two years and three locations, are summarized in Table 1. The mean level of impermeability was much lower at NC than SC or FL, and the genotype x environment interaction effect was highly significant ($P<0.01$). On a mean basis, impermeability in these genotypes ranged from 0 to 78% impermeable seeds at 1 h, and 0 to 57% at 27 h. The variability of impermeability levels across environments is large, which complicates selection for the trait. A genotype with an apparently low level of impermeability at one location may interact with the environment at another location and express a significantly higher level, or vice versa. Such instability in expression also may seriously affect the protective function of the impermeable seedcoat.

As each pair of genotypes traces to an F_5 row (F_4 plant), they cannot be considered true isolines; however, the near-isogenic background of these materials should be useful to reduce the confounding of other effects in impermeability studies. Seed of these genotypes will be increased in 1988. Experiments are planned or in progress to examine the effect of temperature on impermeability expression, to determine the effect of various levels of impermeability on the incidence of seedborne fungi, and to select further in this material for the development of isogenic breeding lines.

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2) Identification of a desirable blend combination in southern soybeans.

Proper management of intergenotypic competition has long been recognized as a mechanism for yield improvement (Borlaug, 1953). That is, multiple genotypes can be grown purposely as a blend so that the components interact to produce a higher farm yield. Yield advantages of blends over purelines have been reported in corn (6%) and wheat (7.6%) (Hoekstra et al., 1985; Rao and Prasad, 1984). Furthermore, most peanut cultivars used in the U. S. today are multiline mixtures.

Although blends of soybean varieties have not gained wide acceptance in the United States, blends may yet find a niche in American agriculture. Recently, Schweitzer et al. (1986) reported that a blend of the Midwestern cultivars 'Elf' and 'Century' yielded 12% more than the pureline component average. This positive result is in marked contrast to those of most blend studies, which depict low blend responses. In the southeastern U. S., few desirable soybean cultivar blends have been identified. The objective of this study was to illustrate the superior performance of a blend derived from two cultivars of Southern maturity.

Materials and methods: This study was conducted at the Central Crops Research Station, Clayton, NC in 1986. The blend of 'Young' and 'Centennial' was chosen on the basis of prior yield performance in eight North Carolina environments. These cultivars are both of Group VI maturity.

Two blending techniques were employed at planting to impose intergenotypic competition within the blend. These were:

1) Randomly blended rows: A 50:50 blend of genotypes planted mechanically in 0.97-m spaced 3-row plots. Genotypes were randomly mixed within packets before planting.

2) Alternated seed hill rows: 50:50 blended genotypes were hand planted in 0.97-m spaced rows marked with a dibble so that each and every hill within a row was bordered by a partner (contrasting) genotype. Hills within a row were spaced approximately 2 cm apart.

Pure lines and blends were treated similarly for a given planting technique. Pubescence color was used as a genetic marker to verify that equal frequencies of genotypes were gained in the blended rows. At maturity, plots were end-trimmed and yield data were taken from 4.5 m of the middle row of each plot. Blends in the alternated hill arrangement (technique 2) were separated into components at harvest using pubescence color as a distinguishing marker.

Statistical analysis: A randomized complete block design with nine replications was used to test overall blending effects on seed yield. Blend components from mixed stands were compared directly to components in pure stands using data obtained from four replications of planting technique 2. Positive blending responses were tested with one-tailed F-tests reflecting the preplanned nature of the comparisons.

Table 1. Mean yield of Centennial/Young soybean blend and components with percentage increase over pureline means.

Genotype	Seed yield[+]	Increase over pureline mean
	kg/ha	%
Young/Centennial Blend	3095	8.0*
Young	2960	
Centennial	2739	
Pureline mean	2849	

[+]Yield values and significance levels based on analysis of variance using a log transformation (LSD not presented for detransformed means).

*significance at 0.05 probability level.

Table 2. Summary of blend components from alternated seed hill planting arrangement in soybean.

Genotype	SDWT	Seed/ha	Yield[+]
	g/100	no. x 10^3	kg/ha
Young, pure stand	13.5 n.s.	23290**	3175**
Young, blend	13.7	28085	3853
Centennial, pure stand	12.5 n.s.	22791 n.s.	2847 n.s.
Centennial, blend	13.0	20946	2724
SEM[++]	0.4	1082	206

[+]Yield of blend components is calculated as (yield/plant) * (total no. plants in a row) * 2.266.

[++]SEM = standard error of a mean.

**Significant difference between pure stand and blend means within a cultivar at 0.01 probability level.

Results and discussion: The Centennial/Young blend yielded significantly higher than the purelines (8.0% above the average of the blend components, Table 1). The consistency of this result with previous trends at eight locations strongly suggests that the observed yield enhancement in the Centennial/Young blend is biologically significant and repeatable.

Components of blend: In blending technique 2, the components of the blend were harvested separately, and thus the competitive role of each genotype could be evaluated. The analysis of component data indicated that the genotype Young yielded 21% more in blend combination than in pure stands (Table 2). By contrast, Centennial yielded approximately the same in mixed and pure stand. [Note: Yield data from components in mixed stand were adjusted to an area basis for comparison with pure stand.] The Young blend component performed markedly better than Centennial in mixed stand. The significantly higher yield of Young within the blend may be attributed to an increase in number of seed per plant because no change in the seed weight was observed. This result indicates that desirable intergenotypic competition could have occurred between Centennial and Young before flower initiation.

Schweitzer et al. (1986) observed a 12% yield increase in a blend of two strikingly diverse genotypes with respect to maturity, height, and growth habit. However, Centennial and Young do not differ markedly in any of these characters. Intergenotypic competition in the present study may thus be the result of an as yet undetermined complex interaction of the two genotypes. It is of interest that the genotype Centennial is resistant to races 1 and 3 soybean cyst nematode. While cyst nematode levels were assumed to be quite low in the fields used for this study, (note that Young, a susceptible cultivar, out yielded Centennial) it remains possible that nematodes play a role in the blend response. Tinius et al. (n.d.) examined the response of the Centennial/Young blend to inoculation with race 1 of soybean cyst nematode. In that study, root and shoot growth of the blend were similar to Centennial in pure stand and much greater than Young in pure stand 60 days after inoculation. It is an unresolved issue that the number of cysts recovered from the blend was very similar to that for Young. Further evaluations of the unique Centennial/Young blend may lead to a better understanding of the intergenotypic competition we have observed. We invite soybean breeders in the South to yield-test this blend in their own fields to assess the blend response over a wider geographic area.

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1) An unstable mutation affecting soybean seed coat color

The **r-m** allele of soybean produces a mottled or variegated seed coat color such that spots and/or concentric rings of black pigmentation are superimposed on an otherwise brown seed coat. We have examined this allele through several generations and found that it undergoes an instability in both somatic and germinal cell layers that causes expression of the R locus to cycle through active and inactive phases. A brief summary of some of these results is presented.

Approximately 30 seed of line L72-2040 (obtained from R. L. Bernard, USDA germplasm collection) containing the **r-m** allele were planted and bulk harvested. A small percentage of all black seed was found, indicating a possible instability in the allele. To eliminate the possibility that the black seed were derived from a stray plant and to verify that mutability of the **r-m** allele was being observed, a large number of mottled seed from the 1983 bulk harvest were planted in our 1985 field plot and individual plants were scored for their seed coat phenotypes. From 544 plants total, 496 (91.2%) produced all mottled seed, 29 (5.3%) produced a mixture of black and mottled seed, 7 (1.3%) produced all black seed, 2 (0.4%) produced brown and mottled seed and 10 (1.8%) produced all brown seed.

Somatic instability of the allele is manifested when individual plants derived from the original stock produced seed mixtures for seed coat color (i.e., black + mottled or brown + mottled). This is unusual, since the seed from a given plant should be uniform in their color because the seed coat is a maternally derived tissue and should reflect the genotype of the parent plant. The change in expression of the allele can occur early or late in development of the plant and there appears to be no consistent pattern of mutability.

The off-type seed from the 1985 field plot were analyzed through two generations of self crosses to determine whether germinal instability was occurring in the **r-m** allele. Representative data are shown in Tables 1 and 2 for some of the off-type plants which produced all black or brown seed. Table 1a shows that the black seeded plants either essentially bred true for the black seed coat or segregated 3:1 for black:mottled seeds. These segregation patterns indicate germinal mutability of the **r-m** allele. Depending on whether one or both of the alleles are affected, plants are produced that are either homozygous **R*R*** or heterozygous **R*r-m** (revertant alleles are highlighted with an asterisk). New off-type plants occur at a low frequency in some of the progeny rows (Table 1a) and represent continued mutability of the allele. Progeny rows of seed derived from some of the individual plants from Table 1a were grown and again the mature plants were scored for their seed coat phenotype (Table 2a). These data demonstrate that the homozygous **R*R***, black seeded progeny from Table 1a

Table 1. Segregation in the first generation of soybean progeny plants derived from the mutable seed stock and revertants

Parent line & phenotype	Total progeny	Seed phenotype of progeny plants			
		Mottled	Black	bl+mot	Brown
(a) 100% black					
RM-83-85-30	66	2*	62	2*	
RM-83-85-31	54		54		
RM-83-85-32	60	17	43		
RM-83-85-33	39	1*	35	3*	
RM-83-85-34	61	13	44	4*	
(b) 100% brown					
RM-83-85-37	66				66
RM-83-85-38	79	1*			78
RM-83-85-39	88		1*		87
RM-83-85-40	111	2*			109
RM-83-85-41	84				84

* represent new germinal or somatic reversion events.

Table 2. Segregation in the second generation of soybean progeny plants derived from the mutable seed stock and revertants

Parent line & phenotype	Total progeny	Seed phenotype of progeny plants			
		Mottled	Black	bl+mot	Brown
(a) From the original 100% black seed stock of Table 1a					
RM-83-85-30					
-2 (black)	45		45		
-3 (bl+mot)	38	2	22	14	
RM-83-85-32					
-3 (mottled)	41	39	1	1	
-4 (mottled)	57	56		1	
-5 (mottled)	41	41			
(b) From the original 100% brown seed stock of Table 1b					
RM-83-85-39					
-2 (black)	36		23	7	6
RM-83-85-40					
-6 (mottled)	9	5			4
-7 (mottled)	22	10			11

(RM-83-85-33, for example) continue to exhibit a low frequency of mutation to plants mixed with phenotypes of black + mottled (i.e., RM-83-85-33-4 or RM-83-85-33-5, Table 2a) indicating that the R* revertants are not completely stable.

Progeny rows of seed derived from those plants producing all brown seed were analyzed (Table 1b). Each plant essentially bred true for the brown seed coat. However, new off-type plants were found to occur at a low frequency in some of the progeny rows (Table 1b) and these were analyzed for a second generation (Table 2b). As shown, the black seeded revertant RM-83-85-39-2 produced a progeny row that segregated roughly 3:1 for black: brown indicating that a single mutation event occurred in the parent (RM-83-85-39) to produce a heterozygous (r*--- R*r*) offspring. Thus, it appears that the newly derived r* form of the allele is still unstable such that it can mutate to R*. Interestingly, some plants with mixed black + mottled seed were also found in the progeny row (Table 2b). This finding again demonstrates the tendency for the mutable gene to frequently interconvert between the three alternative forms (R*, r*, and r-m) both somatically and germinally.

Plants producing a mixture of both black + mottled seed were examined in a similar manner except that the two different seed phenotypes were analyzed separately. The results (data not shown) demonstrated that the black and mottled seed types that occur on the same plant are identical in genotype although they are phenotypically distinct. Therefore, the mutations in the black seed are strictly somatiic and do not reflect mutations in the germ line. However, germinal mutability is also occurring in these lines, as indicated by the occasional off-type plants that were produced. Similar results were found for the original plants that produced a mixture of brown and mottled seed on the same plant.

We have shown that a line carrying the r-m allele can generate somatic and germinal revertants. However, the revertants are not completely stable but can interconvert between all three forms. We propose that a transposable element residing in the r-m allele goes through reversible changes that lead to phenotypic change in expression of the R locus such that the R gene is either expressed (R*) or not expressed (r*). The allelic conversions at the r-m locus are similar to the effects caused by certain maize transposable elements that cycle through active and inactive phases.

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2) The Tgm family of transposable elements in soybean.

An insertion element, Tgm 1 (transposable element, Glycine max), was identified from analysis of a stable recessive mutation in the seed lectin gene (Vodkin et al., 1983; Rhodes and Vodkin, 1985). We have examined six additional Tgm elements that were selected from a lambda genomic library of soybean DNA on the basis of hybridization with subcloned regions of Tgm 1 from the seed lectin gene. These elements ranged in size from 1.6 kilobase pairs to greater than 12 kilobase pairs. Tgm 2, Tgm 3, Tgm 4, and Tgm 5 represent partial isolates in which the genomic clone contained a 3' but not a 5' terminus of the element, while Tgm 6 and Tgm 7, like Tgm 1, were small isolates flanked by both 5' and 3' non-element sequences. Cross-hybridization studies between subcloned portions of these seven elements identified regions of homology, which suggest that the Tgm transposable elements of Glycine max form a family of deletion derivatives. In addition to internal deletion events, numerous deletions and base substitutions are also present within the borders of these elements that are comprised of the same tandemly repeated sequence. The 39% amino acid homology between a 1 kilobase portion of an open reading frame in Tgm 4 and Tgm 5 and ORF1, an open frame from the first intron of the maize Enhancer transposable element, suggests that both elements encode a common function that requires a high degree of protein conservation.

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3) Identification of chalcone synthase and phenylalanine ammonia lyase genes in soybean.

We have cloned soybean genes encoding two key enzymes in the anthocyanin biosynthetic pathway, phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS). The purpose was to obtain molecular probes to study the organization of these genes in soybean and for examination of novel regulatory mechanisms present in the anthocyanin biosynthetic pathway of the seed coat. A soybean genomic library was constructed in the bacteriophage vector lambda Charon 35. CHS and PAL cDNA clones from Phaseolus vulgaris were used in screening the library. Three CHS genes and two PAL genes were isolated. The genes were identified by sequence homology to the CHS gene of Antirrhinum majus (snapdragon) and the PAL gene of P. vulgaris. Both the PAL and CHS sequences of soybean exist as multigene families. The CHS gene family has four to seven members while the PAL gene family consists of only two to three members. The representative CHS gene that was characterized contains at least one intron at the same relative position as intron I of A. majus and no intron corresponding to intron II of A. majus. The probes for CHS and PAL are currently being used to examine the expression of these genes during seed coat development in certain genetic lines carrying alleles of the R and I loci, which affect seed coat pigmentation.

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1) NADH oxidation in roots of three determinant soybean cultivars.

Reduced nicotinamide adenine dinucleotide (NADH) is an important plant metabolite that participates in and provides regulation of many respiratory reactions. It is through the oxidation of NADH that plants obtain energy, exchange electrons, and, as recently revealed in corn (Zea mays L.) roots (Lin, 1982), regulate nutrient ion transport. Coupled together, the direct consequences of NADH oxidation may result in influencing agronomic traits of target crops. In alfalfa (Medicago sativa L.), for instance, quantitative and qualitative levels of NADH have been shown to influence winter hardiness (Krasnuk et al., 1978), but the ability of the crop to withstand cold may actually be a direct effect of those processes originally influenced by NADH oxidation. Like alfalfa, soybean vigor, and possibly yield, may be a direct consequence of NADH metabolism.

Studying enzymes that control NADH oxidation may provide knowledge about why plants differ in their ability to use NADH and what these differences mean to plant performance. This study concentrates on finding soybean cultivar differences in root NADH oxidation and evaluating possible relationships of root enzyme activity to soybean dry matter yield, root pH, and root redox potential.

Materials and methods: Bulk plant source seeds were obtained from E. E. Hartwig at the Delta Branch Experiment Station at Stoneville, MS, and single-plant source seeds were obtained from C. E. Caviness at the Arkansas Agricultural Experiment Station at Fayetteville. Germination took place in an environmental chamber in vermiculite and seedlings were transferred to a nutrient solution at the VC to V1 stage (Fehr and Caviness, 1977). A preliminary experiment evaluated ten cultivars from bulk and single-plant seed sources on the basis of root enzyme activity and total dry matter yield at the V2 to V3 stage. Subsequent experiments focused on finding differences among three selected cultivars from the bulk seed source in root enzyme activity, pH, and redox potential at the V1 to V2 stage.

Plants for the preliminary and selected-cultivar experiments were grown at temperatures ranging from 22 to 27° C during the day and from 19 to 23° C at night. A 14-hr day with a radiant flux density of 20 to 25 W m^{-2} and a relative humidity of 65 to 75% was followed by a 10-hr night with the same relative humidity.

Data from the preliminary and selected-cultivar experiments were evaluated with a randomized complete block design and three repetitions. Combined data from bulk and single-plant seed sources were used in the preliminary experiment and combined bulk seed source data from two replications were used in the selected-cultivar experiment. Analysis of data was performed at the 0.05 level by LSD and at the 0.10 level by Pearson correlation.

In the preliminary experiment, the same amount of fresh root tissue was removed from each plant for enzyme analysis and the remainder was used for dry matter yield. In subsequent selected-cultivar experiments, root tissue was sampled at a younger growth stage and the rest of the plant was discarded. Enzyme assays were essentially the same for all experiments.

Within 2.0 cm of the root tip, 0.10 g of fresh root tissue was removed from each plant at the V2 to V3 stage for the preliminary experiment and at the V1 to V2 stage for the selected-cultivar experiment. Root tissue was rinsed in deionized water, ground with 10 mL of deionized water for 30 sec. in a Ten Broek homogenizer, and placed in a sample vial at room temperature. Redox potential and pH of the tissue solution were measured twice, within 1 hr of sampling, by a redox and a pH electrode connected to a selective ion analyzer. Redox values were adjusted to coincide with the value of the standard hydrogen electrode.

Enzyme preparations were performed after all pH and redox readings were made. Samples were transferred to centrifuge tubes with 5.0 mg of insoluble polyvinylpolypyrrolidone (PVP) and mixed 10 to 20 sec on a vortex mixer to remove phenolic interference. Centrifugation for 10 min at 1200 g was the final stage for obtaining a crude extract for the NADH oxidation assay.

Assays for each crude extract were performed within 3 to 5 hr of the initial tissue sampling. For each sample, 2.0 mL of centrifugate was mixed with 0.10 mL of 1.0mM NADH in a cuvette and placed in an HP8451A Diode Array Spectrophotometer. The NADH was buffered with 1 mM Ultrol Hepes buffer to obtain a pH of 7.0 ± 0.5. Once the cuvette was placed in the spectrophotometer, measurements of absorbance at 340 nm were made at 5 sec intervals for 2 min. Three absorbance-vs-time plots were stored on a diskette and later converted by the first-order kinetic equation to calculate NADH oxidation activity in units of micromoles of NADH oxidized per milligram of fresh root tissue per minute ($\mu M\ mg^{-1}\ min^{-1}$).

Results and discussion: The preliminary experiment showed 'Forrest' and 'Mack' soybeans to have a significantly higher dry matter yield than 'Pershing' soybeans at the V2 to V3 stage (Table 1). The tendency for Forrest soybeans to outperform Pershing soybeans is also reflected in the 1985 variety trials at the Mississippi Agricultural and Forestry Experiment Station (Askew, 1985) and the 1986 variety trials at the Arkansas Agricultural Experiment Station (Walker, 1987). Although there were no significant differences in NADH oxidation among the three soybean cultivars in preliminary experiments (Table 1), there was a tendency of decreasing total dry matter yield with increasing root NADH oxidation activity (Figure 1). This relationship implies that soybean roots may have a lower tendency to oxidize NADH when there is a principle effort to increase dry matter accumulation. Recently, Kishitani and Shibles (1986) showed that a high-yielding soybean cultivar distributed less dry matter to the roots and also had a lower total root respiration than a low-yielding cultivar. This superior yield could possibly result from a well-timed limitation of NADH oxidation potential in soybean roots.

Subsequent experiments with the three cultivars revealed significant differences in NADH oxidation when root tissue from the V1 to V2 stage was used (Table 2). The order of NADH oxidation followed that encountered in the preliminary experiment. From these results, it is apparent that there is a consistent superiority in the ability to oxidize NADH among the three cultivars.

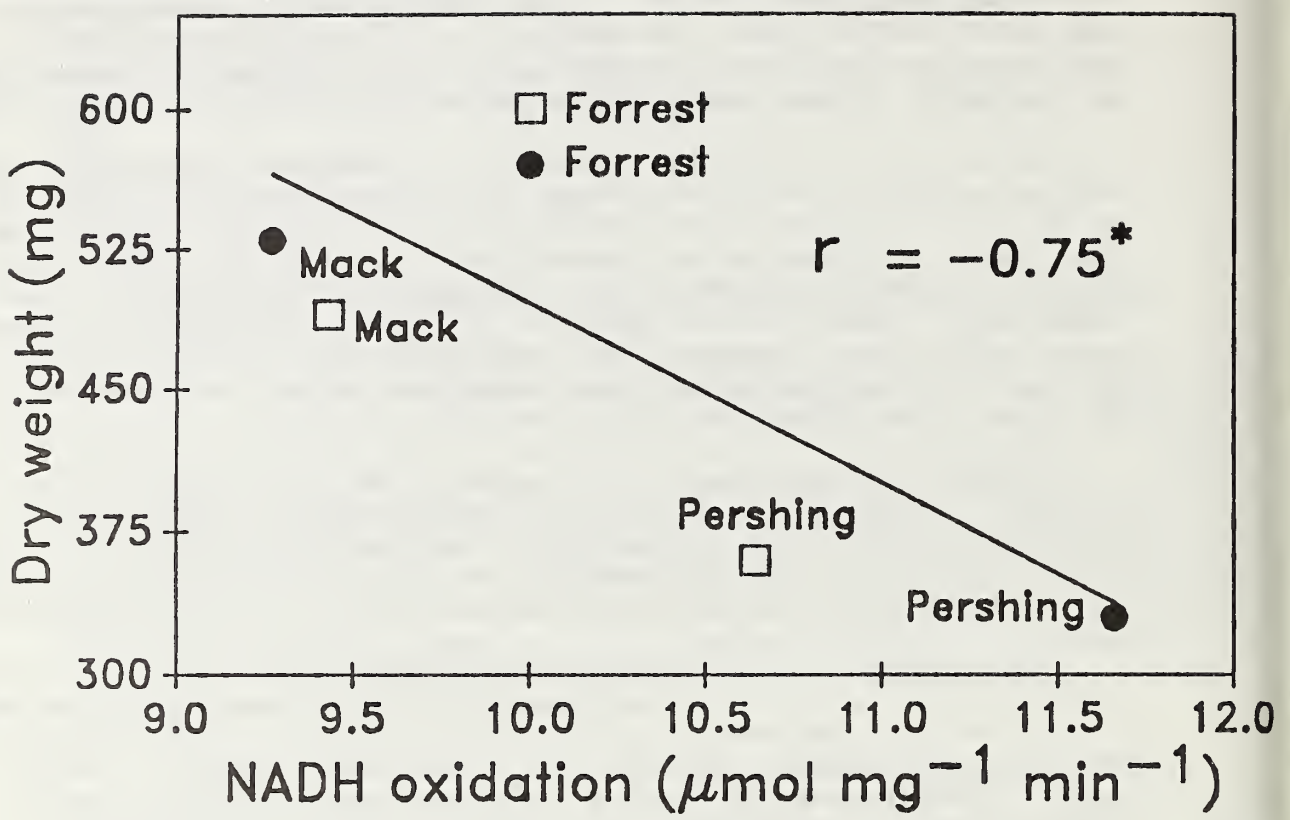

Figure 1. Correlation between total dry matter yield and root NADH oxidation activity for three soybean cultivars at the V2 to V3 stage. Each point represent three plants (|‾| = bulk plant seed source, r = -.571 ● = single plant seed source, r = -0.90). *-significant at p = 0.10.

NADH's close link to respiration enables its relation to respiratory differences among soybean cultivars. Other researchers have identified superior efficiency among genetic lines in winter wheat (Triticum aestivum L.) (Pearman et al., 1981) and ryegrass (Lolium perenne L.) (Wilson, 1982). For the three cultivars studied, we might predict that, although Forrest has the lowest NADH oxidation activity, it has a greater ability to conserve energy and thereby has the respiratory efficiency to enable higher yield potential.

Enzymatic techniques for predicting the seed content (Yabuuchi et al., 1982) and identification (Blogg and Imrie, 1982) have been devised for soybeans. The enzymatic technique evaluated in this NADH oxidation investigation may have wide potential in similar soybean prediction applications because of the numerous metabolic pathways linked to NADH oxidation.

No significant differences in root pH or redox potential were encountered in the selected-cultivar experiment. However, it was noted that there was a tendency for decreasing pH and increasing redox potential with decreasing NADH oxidation (Table 2). Similar negative relationships between

Table 1. Total plant dry matter yield and root NADH oxidation activity for three soybean cultivars at the V2 to V3 stage.

Cultivar	Dry matter yield	NADH oxidation
	(mg)	($\mu M\ mg^{-1}\ min^{-1}$)
Forrest	580 a[1]	9.35 a
Mack	510 a	10.02 a
Pershing	350 b	11.15 a

[1]Means followed by the same letter are not significantly different at the 0.05 level by LSD.

Table 2. NADH oxidation, pH, and redox potential of soybean roots for three soybean cultivars at the V1 to V2 stage.

Cultivar	NADH oxidation	pH	Redox
	($\mu M\ mg^{-1}\ min^{-1}$)	($-\log[H^+]$)	(mV)
Forrest	6.7 a[1]	6.51 a	555 a
Mack	12.3 b	6.46 a	562 a
Pershing	15.2 c	6.43 a	564 a

[1]Means followed by the same letter are not significantly different at the 0.05 level by LSD.

pH and NADH oxidation have been found in oat (Avena sativa L.) roots (Rubinstein et al., 1984) and corn roots (Lin, 1984). Accelerated NADH oxidation at low pH in these plant roots indicates that metabolites consume H^+ and thereby decrease the potential for nutrient ion exchange via H^+ pumping.

In the results presented, it is possible that the negative relationship between NADH oxidation and biomass yield may result from indirect retardation of nutrient ion exchange by NADH oxidation consumption of H^+.

Conclusions: A negative relationship between total dry matter yield and root NADH oxidation has been shown for data combined from three soybean cultivars. Differences in dry matter yield at the V2 to V3 stage may be attributable to differences in NADH oxidation at the V1 to V2 stage encountered among Forrest, Mack, and Pershing soybeans. Other results indicate that there is a relationship between pH and NADH oxidation in the rooots of these three soybean cultivars, which implies a nutrient ion exchange mediated by NADH oxidation.

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1) Identification of the cytoplasmic donor of three semi-wild soybeans.

The conservation mode of evolution of the chloroplast genome of land plants combined with the cytoplasmic inheritance of ctDNA (maternal in most angiosperms) makes the analysis of restriction enzyme fragment patterns a particularly useful tool for understanding evolutionary relationships between closely related species (Curtis and Clegg, 1984).

Shoemaker et al. (1986) demonstrated the existence of both inter- and intra-specific ctDNA diversity within the genus Glycine, subgenus Soja. Five plastome groupings were defined, based upon RFLP patterns. Group 1 (the largest of these) contained exclusively G. max lines, primarily modern cultivars and their maternal ancestors. All of the G. soja accessions were placed into group 3, a diverse group containing primitive G. max lines and G. gracilis as well.

Using a library of mung bean ctDNA clones (a gift of Dr. J. Palmer and Dr. J. Doyle), we probed Southern blots of restriction enzyme digests of total DNA preparations from a sampling of Glycine germplasm. This germplasm screen overlapped the previous study to some extent and also included additional G. max and G. soja cultivars and Plant Introductions.

The patterns of RFLPs from our study confirmed the placing of the modern cultivars together into group 1, but surprisingly, three G. soja Plant Introductions were found to share this same pattern of RFLPs. These are PI 468904, PI 468905, and PI 468906.

PI 468905, PI 468905 and PI 468906 were collected in 1984 by Dr. R. G. Palmer (1981 U.S. Germplasm and Bio-Control Team) from the Gongzhuling Germplasm Nursery, Jilin Academy of Agricultural Sciences, Gongzhuling, Jilin Province, People's Republic of China. These were characterized as semi-wild soybeans, apparently of hybrid origin (Bernard, 1986). The molecular analyses of these G. soja accessions demonstrates that they possess a chloroplast genome uniquely characteristic of Glycine max. Hatfield et al. (1985) showed maternal inheritance of ctDNA in genus Glycine, subgenus Soja. Our ctDNA analyses provide evidence that PI 468904,PI 468905 and PI 468906 are indeed of hybrid origin and, furthermore, that the maternal parent of the outcrossings were Glycine max.

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1) Growth and development of soybean plants under prolonged influence of low temperature.*

Materials and methods: Experiments were carried out in 1982-86 to find cold tolerant soybean genotypes. For this purpose the plants were grown under day and night temperatures of 16°C and 12°C, respectively. The length of light period was 12 hours. In one experiment, day temperature was 17°C, night temperature 14°C. The light intensity in growth chambers was between 25.000 and 30.000 lux. Soybean plants were grown in phytotron before they reached the phase of first leaves, then they were placed in a growth chamber (26°C day temperature, 16°C night temperature during the first 48 hours) and after that temperature was lowered to 16°/12° (day/night). In the course of the vegetation, the state of the plants was systematically evaluated. Each variety was grown in four pots with four plants in each. Soybean plants were grown in climate chambers until early physiological maturity that averaged 111-137 days. In 1982, after the low temperature effect, the pots were placed in the natural conditions to observe the plants' recovery after temperature stress.

Results and discussion: The growth of soybean plants under the low positive temperatures over a long period resulted in high depression, chlorosis of leaves, and dwarfism. In 1982, such varieties as 'Vengerskaya 48', 'Yantarnaya', 'North Record', 'Lanka', 'Kirovogradskaya 4', 'Crest 1', 'Pavlikeny 2', 'Belosnezhka', 'Amurskaya 41', 'Anoka', 'Herb 610', 'Zora', 'Amurskaya 411', 'Grunt', '1SZ-12', 'Beachwood', 'Peremoga', 'Vysokoroslaya 1', 'K-1790', and 'Boby zholtye maslichnye', were included in the experiment. None of them formed pods, though different degrees of chlorosis were observed. At the end of the experiment, the varieties Beachwood, Vysokoroslaya 1, K-1790, and Boby zholtye maslichnye demonstrated the best phenotypic appearance. After placing the pots in natural conditions, the plants of varieties Yantarnaya, Pavlikeny 2, Belosnezhka, Amurskaya 41, 1SZ-12, and Peremoga immediately perished. The varieties Beachwood and Boby zholtye maslichnye formed two beans during the growth period of 30 days in natural conditions, and showed better appearance than others. These results demonstrate that soybean plants are characterized by considerable genotypic variability for the recovery of cold stress damages.

Table 1 indicates the varieties included in the experiment in 1983. It is clear that the Hungarian soybean variety I-1 and the forms Amurskaya 310 and ISZ-7 formed the largest number of pods.

Thus the increased cold resistance of I-1 and ISZ-7 varieties, which was revealed by Schmid and Keller (1979, 1980), has been confirmed in our experiment.

In 1983, the experiment was carried out under less cold stress (17°/14°C). Table 2 demonstrates that Kievskaya 71 and Vengerka show the maximum fruit formation capacity in the present conditions. Varieties Amurskaya 310, I-1, Smena, and ISZ-7 occupy intermediate position. Bucuria, Negrutsa, and Belosnezhka were less tolerant to low temperatures.

Table 1. The average number of formed pods per plant under low temperature conditions.

Variety	Number of pods /plant	Variety	Number of pods /plant
Amurskaya 41	0.14	I-1	1.50
Volna	0.15	Belosnezhka	0.10
Amurskaya 310	0.50	McCall	0.21
ISZ-7	0.40	Maple Arrow	0.27
Kirovgradskaya 4	0.12	Terezinskaya 24	0.10
Bucuria	0.35		

In 1984, a new set of germplasm lines, such as 'North Record', 'Bravella', 'Severnaya 4', 'Timiryazevskaya 122', 'K-309', 'Terezinskaya 2', 'Kievskaya 48', and 'Severnaya 5', was studied. Kievskaya 71 was taken as a check showing high degree of cold tolerance. None of those new forms formed pods, with the exception of Bravella. In the experiment, Kievskaya 71 was also characterized by high level of cold tolerance.

Table 2. Number of pods formed in different soybean varieties grown under cold temperature stress.

Variety	Number of pods /plant	Variety	Number of pods /plant
Amurskaya 310	1.12	Belosnezhka	0.44
Bucuria	0.38	Smena	1.75
I-1	1.00	ISZ-7	1.94
Kievskaya 71	2.38*	Vengerka	2.56*
Negrutska	0.56		

*=slight differences.

Thus, these experiments indicate genotypic variability for cold resistance of soybean plants. Our Soviet variety Kievskaya 71 can be

recommended as a source of cold tolerance for soybean breeding. Studies indicate that cold tolerance of this genotype exceeds that of other varieties, including Swedish and Canadian ones. Moreover, the genotype is characterized by high germination under low temperature conditions (Sichkar, 1981). Varieties such as Hungarian I-1, ISZ-7, and Swedish Bravella, Moldavian form Vengerka and Far Eastern variety Amurskaya 310 also exhibited a fair cold resistance.

An overall appraisal of the results had led to the conclusion that cold tolerance of any genotype is determined by its direct reaction to the given unfavorable factor and also by damage recovery.

There are some hypotheses concerning the behavior of different heat-loving plants under suboptimal temperatures. Some authors consider that decrease of reproductive ability under low temperature conditions results from increase of pollen sterility (Satake, 1976; Sawada and Kinoshita, 1977; Heenan, 1984). It was determined in the experiments with tomatoes that pollen of the plants with different cold tolerance was characterized by different emergence rates under 25°/10°C (Huner and VanHuystee, 1982). According to Levitt et al. (1961), the increased content of SH-groups in the tissues is related to increased cold tolerance.

Sanbuichi (1980) showed that determinate pubescent soybean varieties with large seeds and wide leaflets were more vigorous at early stages of growth. In cold years such varieties over-yielded indeterminate varieties without pubescence with low number of pods.

Thus, genotypic variability of soybean cold tolerance during the vegetative and generative growth is beyond doubt, though the reasons of such phenomena are not known yet.

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